STUDIES ON ORGANOPHOSPHORUS PESTICIDE, DIMECRON INDUCING CHANGES IN THE BRAIN METABOLISM

ABSTRACT

THESIS SUBMITTED FOR THE DEGREE OF

Doctor of Philosophy

IN

CHEMISTRY

BY

S. MASOOD ZAHEER NAQVI

INTERDISCIPLINARY BRAIN RESEARCH CENTER
AND
DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)

1991

ALIGARH MUSLIM UNIVERSITY

INTERDISCIPLINARY BRAIN RESEARCH CENTRE
JAWAHARLAL NEHRU MEDICAL COLLEGE
ALIGARH-202002, INDIA
Phones:- Res (0571) 29329 : Off. 29005

Prof. Dr. Mahdi Hasan
M.B.B.S., M.S. (Honours), Ph.D., D.Sc.
F.R.M.S., F.I.C.S., F.A.M.S., F.I.A.N.S.
DIRECTOR

CERTIFICATE

This is to certify that the research work incorporated in this thesis titled "**Studies on organophosphorus pesticide dimecron, inducing changes in the brain metabolism**" has been carried out by **Mr. S. Masood Zaheer Naqvi** under my guidance at Interdisciplinary Brain Research Centre, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh. In my considered opinion this research work is suitable for submission for the award of the degree of **Doctor of Philosophy** in **Chemistry** to the Faculty of Science, Aligarh Muslim University, Aligarh.

Mahdi Hasan

(Prof. Dr. MAHDI HASAN)
M.S. (Hons), Ph.D., D.Sc.
Supervisor

PHONE : 25515

DEPARTMENT OF CHEMISTRY
ALIGARH MUSLIM UNIVERSITY
ALIGARH—202 002

CERTIFICATE

This is to certify that the research work embodied in this thesis, titled "**Studies on organophosphorus pesticide dimecron, inducing changes in the brain metabolism**" has been carried out by **Mr. S. Masood Zaheer Naqvi** and it is his original work. It has not been submitted for any other degree, either in this or any other University. This research work is suitable for submission for the award of the degree of **Doctor of Philosophy** in **Chemistry**, to the Faculty of Science, Aligarh Muslim University, Aligarh.

Asif Zaman

(Prof. ASIF ZAMAN)
Ph. D.
Co-Supervisor

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENT

It is indeed my great fortune and pride to put on record my humble gratitude from the abundance of my heart, for Professor Dr. Mahdi Hasan, Director, Interdisciplinary Brain Research Centre, J.N. Medical College, Aligarh Muslim University, Aligarh. He not only proposed this study but guided me literally at each and every stage of it. He has been a source of inspiration for me, like a light house, throughout the course of this study. I am extremely grateful to him for spending a lot of his precious time out of his tight schedule, to discuss my problems and enlightening me with his illuminated advice and scholarly suggestions. Without his dynamic supervision and continued help and encouragement, this thesis could never have seen the light of the day. Prof. Mahdi Hasan can easily be ranked the best teacher who has ever tought me throughout my academic career. Besides a dynamic teacher, he is a great human being with rare quality of generous heart and a lot of affection for his students. I am aware of the fact that I have miserably failed to do justice in expressing my eternal gratitude to the academic giant that Prof. Mahdi Hasan is, but I take solace in the belief that others would fail likewise, for it is an impossible task.

I am immensely indebted to my supervisor, Professor Asif Zaman, Department of Chemistry, A.M.U., Aligarh. I have been lucky to get his kind help and encouragement throughout this investigation. He was kind enough to spend his precious time to hear my problems patiently and to spare no pains in seeing me through, with his fruitful suggestions.

I sincerely acknowldge, with thanks, the timely help, encouragement and invaluable suggestions from Professor Sohail Ahmad, Department of Microbiology, A.M.U., Aligarh.

I am extremely grateful to my seniors, Dr. Fakhrul Islam, Dr. S. Saleem Haider, Dr. Tayyaba Qadri, Dr. Yagana Bano, Dr. Naseem Ahmad Khan and Dr. Priti Vadhva. My appreciation is also due to my colleagues Ms. Achla Gupta, Mr. Hyder Farahani and Mr. Mohd Hossain Loffian, for their everyday help and assistance whenever I needed it.

I am particularly indebted to my best friend, Dr. Abbas Ali Mahdi for his continued moral support, help and encouragement throughout this study. In fact he inspired me very much in completing this thesis in time. Thanks are also due to Dr. Sarkar Haider for encouragement and critical suggestions.

Dr. S. Gauhar Alam Rizvi, Department of Statistics, A.M.U., Aligarh deserves my special thanks for his kind help in the all important statistical analysis of the experimental data.

Thanks are also due to Mr. S.M. Hashim and Mr. Syed Mohammad for the beautiful art-work. The help of Mr. S. Mujeer, Mr. Mansoor Ahmad and Mr. Wajid Husain of the Central Photography and Audiovidual Section, and Mr. Shaukat Ali, Department of Anatomy, J.N. Medical College, is also acknowledged.

I am immensely grateful to Mr. Suhail Ahmad and Mr.Owais Ahmad M/S Micro Services Centre, Lal Diggi Road, Aligarh for the meticulous word-processing of this thesis.

The financial assistance from the Indian Council of Medical Research and Council of Scientific and Industrial Research, New Delhi, in the form of Research Fellowships to me, is gratefully acknowledged.

Finally, I wish to express my heartfelt and deepest gratitude to my parents and sisters for their ever lasting moral support, affection, patience and forebearance, without which it could have been impossible for me to accomplish the ardous task of completing this thesis.

S. Z Naqvi

(S. MASOOD ZAHEER NAQVI)

ABSTRACT

The wide-spread use of organophosphate (OP) pesticides has been found to improve the agricultural out-put, besides controlling the vectors of several parasitic diseases, such as malaria, filaria, etc. OP pesticides are preferred among other pesticides due to their rapid biodegradability. However, indiscriminate and ever-increasing application of OP pesticides has been reported to induce various toxicities to man and other non-target organisms. Brain is the most susceptible and vulnerable organ of the body, and hence attacking the nervous system is the easiest and surest way of chemically upsetting the body metabolism. Generally, most of the organophosphates are referred to as 'anticholinesterases' as they inhibit the acetylcholinesterase (AChE), resulting in the accumulation of acetylcholine (ACh) at the synapses, and thereby leading to disruption of nerve function. ACh accumulation also stimulates cholinergic excitation which,,in turn, may deplete the monoamine neurotransmitters. Monoamine oxidase (MAO) is the main enzyme which degrades the monoamines. Changes in the amine concentrations have been found to produce various behavioural dysfunctions. Brain is one of the most lipid-rich regions of the body. Lipid peroxidation is an important lipid-chain degradation process. Nucleic acids and proteins are also important constituents of brain. Sulfhydryl groups and enzymes glutathione-S-transferase (GST) and superoxide dismutase (SOD) are related

to the detoxification process against the toxicants, e.g. organophosphates.

The present study embodies neurotoxic effects of dimecron, a potent and widely used organophosphate, on AChE, monoamines, MAO, open field behaviour, lipid metabolism, lipid peroxidation, sulfhydryl groups, GST, SOD, nucleic acids and protein contents of albino rats . Efforts were also undertaken to detect the dimecron fractions from the CNS regions of intoxicated rats. Also, protective effects of antioxidant, acetylhomocysteine thiolactone (cithiolone) on the adverse effects of dimecron, were studied.

Open field behaviour was studied following the method described by Ali et al (1980). Enzymes AChE and MAO were assayed by the procedures of Ellman et al (1961) and Tabor et al (1953), respectively. Monoamines, were determined by the method of Welch and Welch (1969). Total lipids, phospholipids, cholesterol, gangliosides and sulfhydryl groups were estimated by the procedures of Woodman and Price (1972), Marinetti et al (1962), Henly (1957), Pollet et al (1978) and Sedlak and Lindsay (1968), respectively. Rate of lipid peroxidation was determined by the procedure of Utley et al (1967). Activities of GST and SOD were studied by the methods of Habig et al (1974) and Marklund and Marklund (1974), respectively. Nucleic acids DNA and RNA were estimated following the methods of Burton (1956) and Dische

(1955), respectively, while the total proteins were determined by the procedure of Lowry et al (1951). Contents of dimecron were detected from the CNS by thin-layer chromatography followed by spectrophotometric method of Anliker and Menzer (1963), modified by Naqvi and Hasan (1990). Significant findings of the present study are summarized as follows:

[A]. Dimecron intoxication (2.0 mg / Kg b. wt, ip) for seven days, was found to produce the undermentioned results:

- A gradual daily decrease was observed in the open-field behaviour parameters, i.e. ambulation, rearing and preening. The greatest decrement was noticed on the seventh day of injection.

- The activity of AChE was inhibited significantly in all the regions of the CNS, viz. cerebrum, cerebellum, brain stem and spinal cord. Maximum depletion was found in the spinal cord.

- Levels of monoamines viz. dopamine (DA), norepinephrine (NE) and serotonin (5-HT) were found to be depleted significantly in all the CNS regions. Maximum decrement of DA was noticed in the cerebellum, while NE and 5-HT were maximally decreased in brain stem and cerebrum, respectively.

- Significant elevation was observed in the MAO activity of all the CNS regions. Cerebellum showed maximum increment while minimum increase was noticed in the brain stem.

- Lipid contents were found to diminish in various CNS regions. Total lipids and phospholipids decreased in all the regions of the CNS with maximum depletion being noticed in cerebrum. Cholesterol was found to deplete in cerebrum, cerebellum and spinal cord. Maximum decrement of cholesterol was however, detected in the cerebrum. Significant diminution in the level of gangliosides was observed in cerebrum, cerebellum and brain stem.

- Rate of lipid peroxidation enhanced considerably in various region of the CNS.

- Content of total, non-protein bound (NPB) and protein-bound sulfhydryl groups were found to decrease significantly in different CNS regions. Spinal cord showed maximum decrement of total and NPB sulfhydryl (-SH) groups while protein-bound -SH groups depleted most significantly in the cerebrum.

- Significant inhibition of GST activity was observed in all the CNS regions. Spinal cord revealed maximum GST inhibition.

- Activity of SOD also depleted in alla the regions of the CNS with maximum depletion occurring in spinal cord.

- The concentration of DNA was found to decreased considerably in all the CNS regions. Maximum decrement was detected in the cerebrum.

- Levels of RNA increased in cerebrum, cerebellum, brain stem and spinal cord with the maximum increment discernible in the spinal cord.

- Protein levels were found diminished in all the CNS regions. Most significant diminution of proteins was noted in the cerebrum.

[B] When acetylhomocysteine thiolactone (cithiolone) was administered (8.0 mg/Kg b. wt, ip) simultaneously with dimecron, remarkable protection was observed against the dimecron-induced adverse effects on open field behaviour, AChE, MAO, lipid peroxidation, and GST and SOD activities in all the regions of the CNS.

[C] Dimecron was recovered from various CNS regions of the rats intoxicated with three graded doses of dimecron (1.0, 1.5 and 2.0 mg /Kg b. wt, ip x 7 days). A remarkable dose-related elevation was observed in the concentration of dimecron accumulated in cerebrum, cerebellum, brain stem and spinal cord. Cerebrum showed maximum percent recovery compared to the concentration of dose injected.

It can be concluded from the present study that dimecron intoxication adversely perturbs the open field behaviours and induces alterations in the neurochemical metabolism, specially ACh, monoamines, lipids, nucleic acids, proteins and related enzymes. Also, antioxidant cithiolone protects against the dimecron neurotoxicity. Moreover, the accumulation of the dimecron in the CNS regions, reported for the first time, supports the view of earlier workers that organophosphates probably induce neurotoxicity by readily penetrating the blood-brain barrier.

1. INTRODUCTION

One of the most important and concerning problems faced by the humanity is that of population explosion. Providing food to ever increasing millions of mouths all over the globe has become a hard nut to crack. Besides, man has also to combat the menace of pests which not only destroy the crop, but act as vectors of some deadly parasitic diseases, e.g. malaria, filaria, etc.

Crop protection by means of chemicals has become increasingly an object for public discussion ever since Rachal Carson published her book "Silent Spring". According to the understanding of the basic concepts involved in this difficult scientific field, two lines of argument follow which tend to be ideological or of a more objective nature. One may regard chemical crop protection simply as a profit-induced poisoning of the environment or one can try to analyse the various factors that have compelled us to use chemical agents in the food production. The deciding factor is, of course, the development of world population.

During the last four five years, there has been a vast increase in variety and consumption of pesticides used in order to improve agricultural yields and preserve harvested crops. The use of pesticides is steadily increasing so much so that the requirement for 1975-76 was twice as that of 1971-72, and in 1983-84 it increased two and half folds.

The word "pest" includes widely divergent group of organisms e.g. insects, rodents, weeds, fungi, molluscs etc. 'Pesticides", therefore comprise of a large variety of compounds of diverse chemical nature and biological activity grouped together, only on the basis that they are used to destroy or eliminate pests. Some of the classifications are based on (a) the class of pest against which they are used, (b) source from which they are obtained (c) based on combination of biological activity and chemical structure. The last mentioned is the most commonly used criterion of pesticide classification, and the pesticides may be classified as Insecticides, Weedicides, Rodenticides, Fungicides, Fumigants, Acaricides, Nematocides and Molluscicides (Table 1.1).

Pesticides are used (a) in agriculture for protection of crops, (b) in public health programmes, against the control of disease vectors e.g. malaria, typhus, yellow fever, filaria, etc., (c) for protection of material, incorporation in paints, timber, etc. (d) in industry, for the control of hazardous vegetation in forests, airports, factory sites, and fumigation of ships etc. (e) domestic use as household and garden spray and control of ecto-parasites in pet animals, and (f) as an application to clothings skin and for the control of ecto-parasites e.g. fleas, lice, mites, etc.

Pesticides are only effective if they are toxic to some forms of life. As a number of pesticides are proved

TABLE 1.1

CLASSIFICATION OF PESTICIDES

Pesticides	Examples
(A) Insecticides:	
i) Organophosphate	Phosphamidon, Dichlorvos, Methyl parathion, Metasystox, TEPP
ii) Organochlorine	DDT, Endosulfan, BHC, Aldrin, Chlordane
iii) Organochlorine	Carbaryl
iv) Botanical	Pyrethrine, Nicotine
(B) Weedicides:	Paraquat, Dalapon, Nitrofen
(C) Rodenticides:	Ammonium sulphate
(D) Fungicides:	Zinc phosphate, Warfarin
(E) Fumigants:	Copper sulphate, Copper oxychloride, Sulfur dust, Dicofol, Ethion
(F) Acaricides:	Alluminium phosphate
(G) Nematocides:	Metham-sodium, Fensulfothion
(H) Molluscicides:	Metaldehyde

indubitably poisonous to people exposed to them, there is a natural concern that their extensive use in food production could result in a health hazard to those who ultimately consume food which at some stage has been exposed to one or more pesticides. Pesticides have been shown to affect adversely a number of biological functions such as nervous system in many organisms, reproduction in birds and fish, hormonal balance in mice, the temperature regulating mechanism in fish, liver functions in man, etc. (Gupta 1975).

1.1.0 Organophosphates:

Organophosphate (OP) compounds are widely used as potent contact and systemic pesticides in agriculture, household and public health programmes. The consumption of organophosphate pesticides has sharply increased in recent years. A majority of organophosphate pesticides in use are basically of a single chemical nature and are the derivatives of phosphoric acid. They are preferred among other pesticides due to their rapid biodegradability as well as penetrant and volatile properties (Moriarty 1975). Besides improving the crop production, some organophosphates have also proved helpful in controlling vectors of certain deadly diseases, such as malaria, filaria, leishmaniasis (Casida and Baron 1976).

The use of organophosphates as agricultural chemicals is not restricted to insecticides. Some of the organophosphates also serve as acaricides, fungicides,

anthelmintics, nematocides, cotton defoliants and crabgrass control agents (Casida 1964). Some OP compounds have also been used medically for treatment of diseases such as myasthenia gravis and glaucoma. But because of narrow gap between therapeutic and toxic doses, this use is not recommended. Unfortunately, during World War II, a group of most toxic OP compounds, "nerve gases" (Soman, Sarin, Tabun) were successively used by Germans as chemical weapons.

Generally, most of the organophosphates are frequently referred to as "esterase inhibitors" or "anticholinesterases" since they inhibit carboxylic esterase enzyme, acetylcholinesterase (AChE) resulting in the accumulation of acetylcholine (ACh) at the synapses, and hence lead to disruption of nerve function (Johnson 1976). Indiscriminate and ever-increasing consumption of OP pesticides has led to severe health hazard to man and other non-target organisms. Numerous incidents of OP poisoning have been reported all over the world. Statistics for 1969 indicate that 50-80% of poisons detected in medicolegal cases in Punjab (India) belonged to organophosphate and organochlorine compounds (Bami 1971). Moreover, Tabershaw and Cooper (1966) observed that acute OP toxicosis degrades axons due to the interference of organophosphates in the regular metabolism of nerve cell bodies. Recently, Naqvi and Hasan (1990) have reported dose-related accumulation of organophosphate pesticide, dimecron, in various regions of the central

nervous system, which suggests that organophosphates cross the blood-brain barrier.

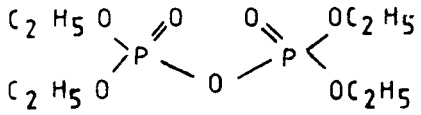
From our laboratory, a number of studies have revealed various neurobiochemical alterations following exposure to organophosphates, dichlovos (Ali and Hasan 1977, Vadhva & Hasan 1986), metasystox (Tayyaba & Hasan 1985) and methyl parathion (Hasan & Khan 1985, Khan and Hasan 1988).

1.1.1 Historical Overview:

Since the dawn of the civilization, man has been fighting a never-ending battle against the army of pests. Search for powerful pest controlling chemicals led to extensive studies all over the world.

Barely 170 years ago, Lassaign (1820) reacted alcohol with phosphoric acid in a reaction analogous to that with sulphuric acid and therewith launched the chemistry of organophosphorus compounds. It was followed by a number of studies with an aim to prepare organophosphate compounds possessing insecticidal properties.

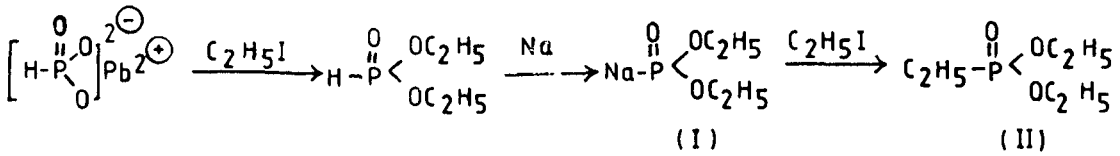
In 1854, Clermont synthesized tetraethyl pyrophosphate (TEPP) by alkylating the silver salt of pyrophosphoric acid with alkyl halides (Clermont method). Clermont (1854), however, did not recognize the important physiological activity of this compound. TEPP was later found to be a potent, anticholinesterase pesticide (Casida 1964).



This ester, which may be regarded as a link between inorganic and organic chemistry, was often described in the following decades and yet almost 80 years were to pass before its insecticidal properties were discovered.

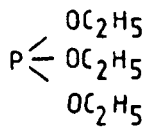
C.A.A. Michaelis (1894-1913) in Germany and A.E. Arbusov (1906-1964) in Russia can be named as the founders of classical phosphoric ester chemistry.

In 1897 Michaelis and Becker reacted sodium dialkyl phosphite with ethyl iodide according to the following scheme:

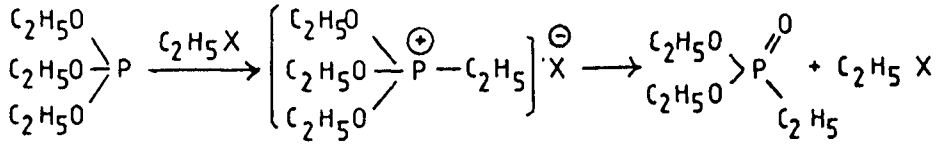


(I) (II)

The reaction of sodium salts of dialkyl phosphites (I) with alkyl halides became known as the Michaelis-Becker reaction and the compound so obtained, a phosphonic ester (II) was not identical with the phosphorous acid ester (III).



which they synthesized from phosphorus trichloride and sodium ethylate. In 1898, Michaelis and Kaehine isolated a compound from trialkyl phosphite and methyl iodide, whose structure they did not recognize as being analogous to (II). The problem was taken up again seven years later by Arbusov in 1906 who repeated the reaction of phosphorus trichloride with sodium ethylate. He succeeded in reacting a highly purified triethyl phosphite (III) with ethyl iodide at room temperature by the following method:

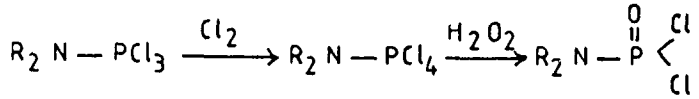


(III)

SCHEME 2 (ARBUSOV REACTION)

Scheme 2 (Arbusov Reaction)

In 1903, Michaelis synthesized phosphorus-nitrogen compounds from phosphorus trichloride, pentachloride, phosphorylchloride, thiophosphorylchloride and ammonia or amines. In the reaction of phosphorus trichloride with alkyl amines he obtained N-alkyl amino-dichlorophosphine (V), which he oxidized with chlorine to the tetrachlorides (VI).



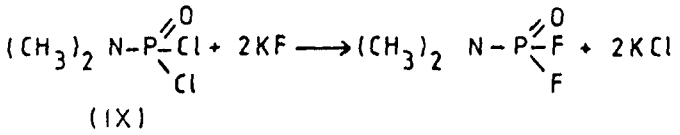
(V) (VI) (VII)

Atmospheric moisture sufficed to hydrolyse these tetrachlorides to dialkylaminophosphorodichloridates (VII). Also, in the reaction of phosphoryl chloride with aliphatic amines, he found R_2NPOCl_2 (VII) in addition to phosphorodiamidochloridate (VIII), an important starting material for phosphorylation reaction.

In 1917, Balareff published several papers concerning the action of phosphoryl chloride on methyl and ethyl alcohol but the final structure of pyrophosphoric acid was not clarified until 1930 by Nylen who established the symmetric form. He synthesized the tetra-alkyl pyrophosphate and mixed pyroesters.

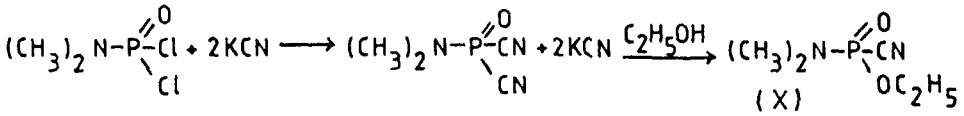
In 1932, Lange and Krueger, working in Berlin, prepared the esters of monofluorophosphoric acid from its silver salts with alkyl iodide. They were the first to draw attention to the highly toxic properties of these compounds, including respiratory distress, clouding of consciousness, temporary blindness and photophobia. Biological and chemical studies on organophosphate toxicants during the following decade were largely restricted to German laboratories. Schrader (1935-1970) been working on acid fluorides in search for compounds with ascaricidal and aphicidal activity. In this field he was first successful with the methane sulfonyl fluoride (CH_3 SO_2 F) in 1946, which is still used today in special cases as a fumigant. By changing from sulfuric acid to phosphoric acid

he was led to what became later his main work. As starting material he used N, N - dimethyl phosphoroamidodichloridate (IX) which is easily convertible to difluoride.



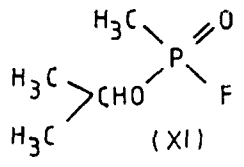
(IX)

At first he found only weak insecticidal properties until he reacted the dichloride with potassium cyanide (Schrader 1937 and 1952), which resulted in the highly toxic and miotic Tabun (X) (Schrader and Gebhardt 1939 and 1953).

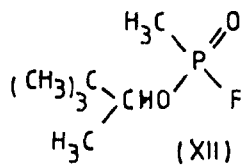


(X)

Then he replaced the dialkylamino group by an alkyl group (Collomp 1949) and so arrived, in 1937, at the pysiologically extremely potent compound, Sarin (XI). Soman (XII), however, did not originate from Schrader, but was synthesized in 1944 in Heidelberg by commission of the "Heereswaffenamt" (Riser 1950). Due to their high mammalian toxicity, neither Sarin nor Soman were used as insecticides (Lohs 1967).



(XI)

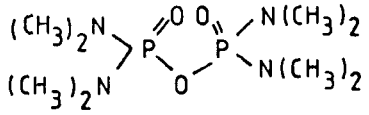


(XII)

During World War II, Tabun, Sarin and Soman, collectively known as "nerve gases" were successfully used by Germans as potential chemical warfare.

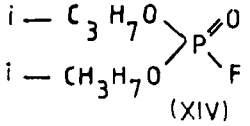
Dimethyl phosphoroamidodichloridate served Schrader (1946) as the key substance for the synthesis of octamethyl pyrophosphoro- amidate (XII), which is a potent systemic insecticide.

In honour of Schrader, scientists of Pest Control Ltd., in 1950 named OMPA as "Schradan".



By reacting phosphoryl chloride with triethyl phosphate, a reaction which has become known as "Schrader process", Schrader (1939/1943) synthesized tetraethyl pyrophosphate (TEPP).

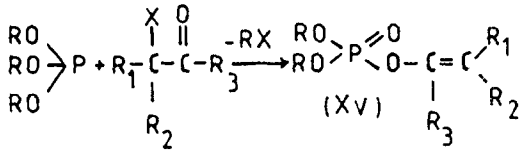
In 1941, Adrian, Felberg, Kilby (1947), and Wirth (1949) discovered the cholinesterase inhibiting action of the organophosphates. This was occasioned by an attempt to clarify the miotic action of DFP (XIV).



(XIV)

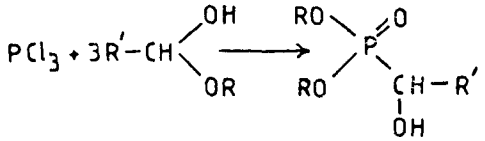
In 1952-54 W. Perkow (Perkow et al 1952; Perkow 1954) described the common reaction of -halogen carbonyl compounds with triethyl phosphite and was the first to formulate correctly the end products as dialkylvinyl phosphates (XV).

The reaction in the following equation has, therefore, become known as the Perkow reaction:

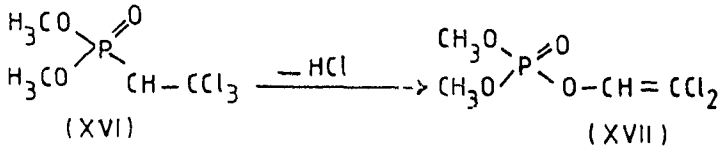


Historically, the enol phosphates were discovered by several teams of researchers at almost the same time. In the first papers, the structure was presumed to be an -ketophosphonate, probably under the influence of the results published by Arbusov et al.

Mattson et al (1955) reported the occurrence of dichlorvos (DDVP) as a by-product of dipterex (trichlorfon). Rosin and Haus (1956/59) claimed a new process for the synthesis of trichlorfon and described the alkaline rearrangement of trichlorfon to dichlorvos.

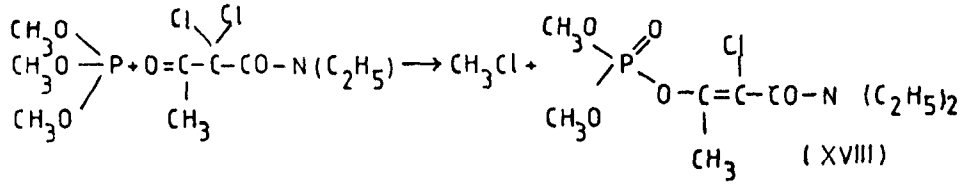


A different route was discovered by Lorenz (1954/57) who showed that trichlorfon (XVI) is hydrolysed to dichlorvos (XVII).



Phosphamidon (XVIII), a member of the class of enol phosphates or vinyl phosphates, was first prepared by E.

Beriger in 1955 by condensing -halocarbonyl compounds with trialkyl phosptites, according to the Perkow reaction

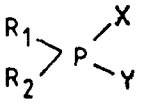


(XVIII)

It has been estimated that over 50,000 organophosphorus compounds have by now been synthesized and screened for their insecticidal potency, of which over 3 dozen have been produced commercially (Chadwick 1963).

1.1.2. General Structure and Nomenclature :

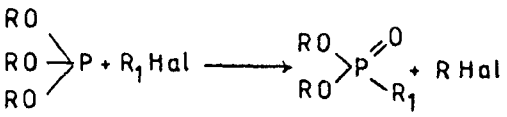
Schrader proposed a type formula for insecticidal organophosphates in 1937:



where groups R_1 and R_2 may be alkoxy, alkyl, aryloxy, aryl, or substituted amino groups. X contains oxygen or sulphur, and the acyl group (Y), is a good leaving group; consisting of fluoride, cyanide, pyrophosphate, substituted phenoxy, thio-alcohols, aliphatic and heterocyclic enoles etc.

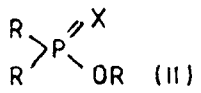
A simple, logical and generally accepted nomenclature of the organophosphorus compounds does not exist. Tradition amongst individual teams plays a great role, as is apparent from a comparison of the Russian (Arbusov) and German (Michaelis) schools.

The type I compound resulting from the Arbusov reaction



(I)

is designated phosphinic acid ester by some Russian authors. In Germany, on the other hand, the compound is described as phosphonic acid ester, and phosphinic acid derivatives are understood to be compounds with two P-C bonds:



(II)

This often resulted in several chemical and trade names for each compound. Fortunately, the 1952 agreement between England and United States of America on the nomenclature of organophosphates is now followed by most of the scientists. In this system, esters are named as derivatives of the corresponding parent compounds. A 'phosphate' is a derivative of phosphoric acid, a 'phosphonate' a derivative of phosphonic acid. Esters containing a nitrogen, sulfur or halide attached to phosphorus have appropriate suffixes. The structures of the organophosphate derivative base names have been illustrated in Table 1.3. Of these simple derivatives, there can be numerous combinations such as phosphonothionates, phosphorodiamidofluoridates, vinyl phosphates, etc.

TABLE 1.2: DERIVATIVE BASE NAMES OF OP COMPOUNDS

Phosphate	$(RO)_3$ P=0
Phosphonate	(R) (RO_2) P=0
Phosphinate	$(R)_2$ (RO) P=0
Phosphoramidate	(RNH) $(RO)_2$ P=0
Phosphorothionate	$(RO)_2$ P=0
Phosphorothiolate	(RS) $(RO)_2$ P =0
Phosphorofluoridate	(F) (RO), R=0

Once the derivative-base name is established, the R-substituent groups are included in the name with an indication of the atom to which they are attached. The structural formulae, chemical names and common names of several representative organophosphates are given in Table 1.3.

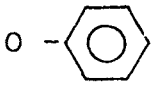
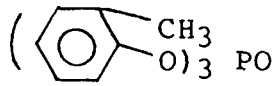
The multiplicity of organophosphates frequently causes them to be victims of confusion between common and proprietary names. A proprietary name is the one usually allocated by the manufacturing company which owns the compound. It should always be used with a capital letter, and is often followed by (R) to show that it is a registered trade mark.

1.1.3 Structure Activity Relationship (SAR):

Repeated references have been made to "Schrader's rule", linking structure and biological activity of the

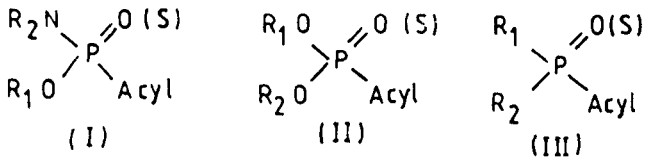
TABLE 1.3

REPRESENTATIVE ORGANOPOHSPHORUS COMPOUNDS

Common Name	Chemical Name	Structural formula
Phosphamidon	2-Chloro-2-diethylcarbamoyl-1-methyl vinyl dimethyl phosphate	(CH_3O) P (O) OC (CH_3) C (Cl) CON $(C_2$ H5$)_2$
Parathion	Diethyl-4-nitrophenyl phosphoro thioate	$(C_2$ H_5 O$)_2$ P (S) - O -  - NO_2
DFP	Diisopropyl phosphorofluoridate	$[(CH_3)_2$ CHO$]_2$ P(O) F
Dichlorvos	2,2-dichlorovinyl dimethyl phosphate	$(CH_3O)_2$ P (O) (OCH C Cl_2)
TEPP	Tetracthyl pyrophosphate	$(C_2$ H_5 O$)_2$ P (O)-O-(O) P $(C_2$ H5$)_2$
TOCP	Tri-ortho-cresyl phosphate	

organophosphates. Schrader (1952) gave the Schraders' empirical rule, according to which biologically active phosphoric acid esters must possess an "acyl" moiety, and it was the first indication that the chemical mechanism of insecticidal action might depend upon the phosphorylation of biologically important targets.

In 1937/38 Schrader gave a specific formulation (I, II) and in 1950 a somewhat more generalized form (III) of organophosphates



In 1963 he described his formula (II) as follows:

"It is likely that a biologically active phosphoric acid ester will be obtained when the following pre-requisites are satisfied: Either sulphur or oxygen must be directly bound to the pentavalent phosphorus, R_1 and R_2 may be alkoxy, alkyl groups or amines while the 'acyl' may be represented by anions of organic or inorganic acids, such as fluorine, cyanate, thiocynates, enolates, mercaptides, etc."

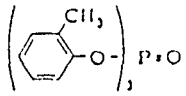
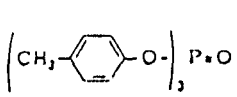
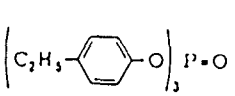
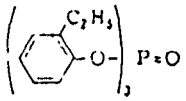
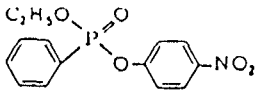
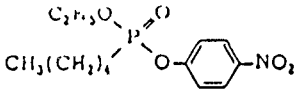
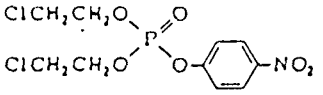
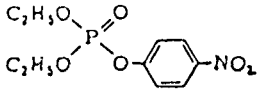
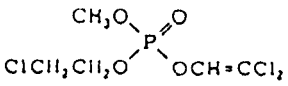
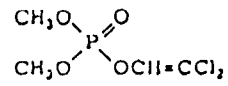
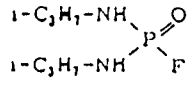
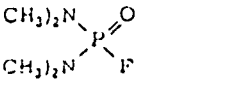
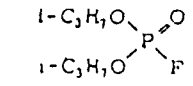
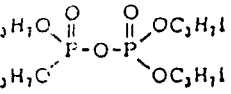
Numerous substantial arguments were put forward showing that the biological activity of organophosphates is to be considered as an inhibition of cholinester-splitting and other serine enzymes, which chemically undergo

phosphorylation of serine-alchohol group at the esteratic site. The dependence of the inhibitory effect both upon the affinity of the inhibitor for the active centre of the enzymes as well as upon the phosphorylation constants means that, due to the complex nature of the inhibition potential, it is only in very favourable cases that there is a direct correlation between the inhibition and phosphorylation action.

A 'Phosphorus inhibitor' contains three types of groups: (i) The basic or neutral 'R' group, (ii) the acidic, acyl group (X) and, (iii) the atom from sixth period of the periodic table, usually oxygen or sulphur, doubly bond to phosphorus. Each type of the group has a typical effect on the activity. Slight structural changes greatly alter the spectrum of biological activity. The activity is determined by the magnitude of electrophilic character of the phosphorus atom, the strength of the P-acyl bond and the steric effects of the substituents.

(i) Basic groups: Change in the basic groups produces a complex variation in 'rates of inhibition', and this variation is different according to whether the enzyme inhibited is an AChE or ChE. There is no general relationship between the rates at which compounds are hydrolyzed and the rates at which they inhibit ChEs, because the effects of the basic groups on rates of hydrolysis are mainly 'electronic' and on rate of inhibition, mainly 'steric'.

Table 1·4 : Structure and neurotoxic action according to Aldridge and Barnis (1967).

Neurotoxic	Non-neurotoxic
	
	
	
	
	
	
	

(ii) Acidic groups: When changes in structures are confined to the acidic acyl group, X, there is generally close correlation between 'rates of hydrolysis' and 'rates of inhibition'. Rates of hydrolysis depend primarily upon the strength of acidic HX. Exceptions are cyanidates, thiolates and phosphate ester containing vicinyl double bonds (P.O.C:C) which hydrolyse abnormally rapidly.

(iii) P.O. and P:S Groups: Thionates are isomerized or oxidized readily to more active compounds. The PI_{50} of 'parathion' is thus about 4 times less than that of its oxygen analogue, 'paraxon'. Perhaps the P:O oxygen in phosphates is hydgrogen bonded to the enzyme surface and this facilitates the reaction. Sulphur has much less ability to form hydrogen bonds, so the thionates react more slowly. Interestingly, the thiono-analogues of ACh and related esters are not hydrolyzed by AChE and other esterases (Bergmann et al 1958).

Biological activity of organophosphates, thus, vary with the slight structural changes even in the same group, as shown in Table 1.4.

1.1.4 Mechanism of action of organophosphates

The central nervous system (CNS) is one of the most susceptible and vulnerable regions of the body. Attacking the CNS is supposed to be the most rapid and sure way of

disturbing the normal body mechanisms. The biological action of most of the organophosphate pesticides on mammals and insects is generally exerted by attacking the neuronal transmission system. The insecticidal action of organophosphates in vertebrates appears to result primarily from the disruption of nerve impulse transmission in the central and peripheral nervous system following inhibition of acetylcholinesterase (AChE) leading to accumulation of cholinergic neurotransmitter acetylcholine (ACh) at the synapses (Johnson 1976).

1.1.5 Molecular mechanism of action

The most significant molecular mechanism of action of most the organophosphates is regarded as their action through phosphorylation of the active centre of cholinesterase (Jaques & Bein 1960).

The unit structure of AChE is a protein with a molecular weight of 80,000. These units are grouped as tetramers (Dudai et al 1973; Rieger et al 1973). The active surface of the enzyme consists of two sites, an anionic site and an esteratic site (Wilson and Bergman 1950). At the anionic site (negatively charged, probably due to free carboxyl group of dicarboxylic acid), the positively charged quaternary nitrogen atom of ACh is attracted by electrostatic forces. The esteratic site has essentially two components located at 2.5, and 5 A^{o}, respectively, from the anionic site: a potentially acidic group (the hydroxyl group of

serine) and a basic nucleophilic group (the imadizole group of histidine). The imidazole group, by hydrogen bonding, enhances the nucleophilic activity of the serine hydroxyl group, thus enabling it to interact with the carbonyl carbon atom of ACh. A covalent bond is formed, with the production of an acetylated-enzyme intermediate and the release of choline. The electrophilic carbon atom of the acetyl group then undergoes nucleophilic attack by the electronegative oxygen atom of a water molecule. The acetyl-enzyme complex is thus hydrolysed, producing regenerated enzyme and acetic acid (Wilson 1951; 1954). A diagrammatic representation of the reaction of ACh with AChE is depicted in Figure 1.1.

The active organophosphate pesticides act by blocking acetylcholinesterase. This inhibition results in accumulation of ACh at the post-synaptic membrane so that it becomes unable to return to its original (resting) state, and thus prevents the smooth transmission of nervous impulses across the synaptic cleft, resulting in the loss of muscular coordination, induction of convulsions and ultimately death.

The reaction between AChE and most of the OPs occurs only at the esteratic site, but proceeds in the manner comparable to the reaction between AChE and ACh (Fig.1.1). If the resultant phosphorylated enzyme is extremely stable: if the attached alkyl groups are methyl or ethyl, significant regeneration of the enzyme by hydrolytic cleavage requires

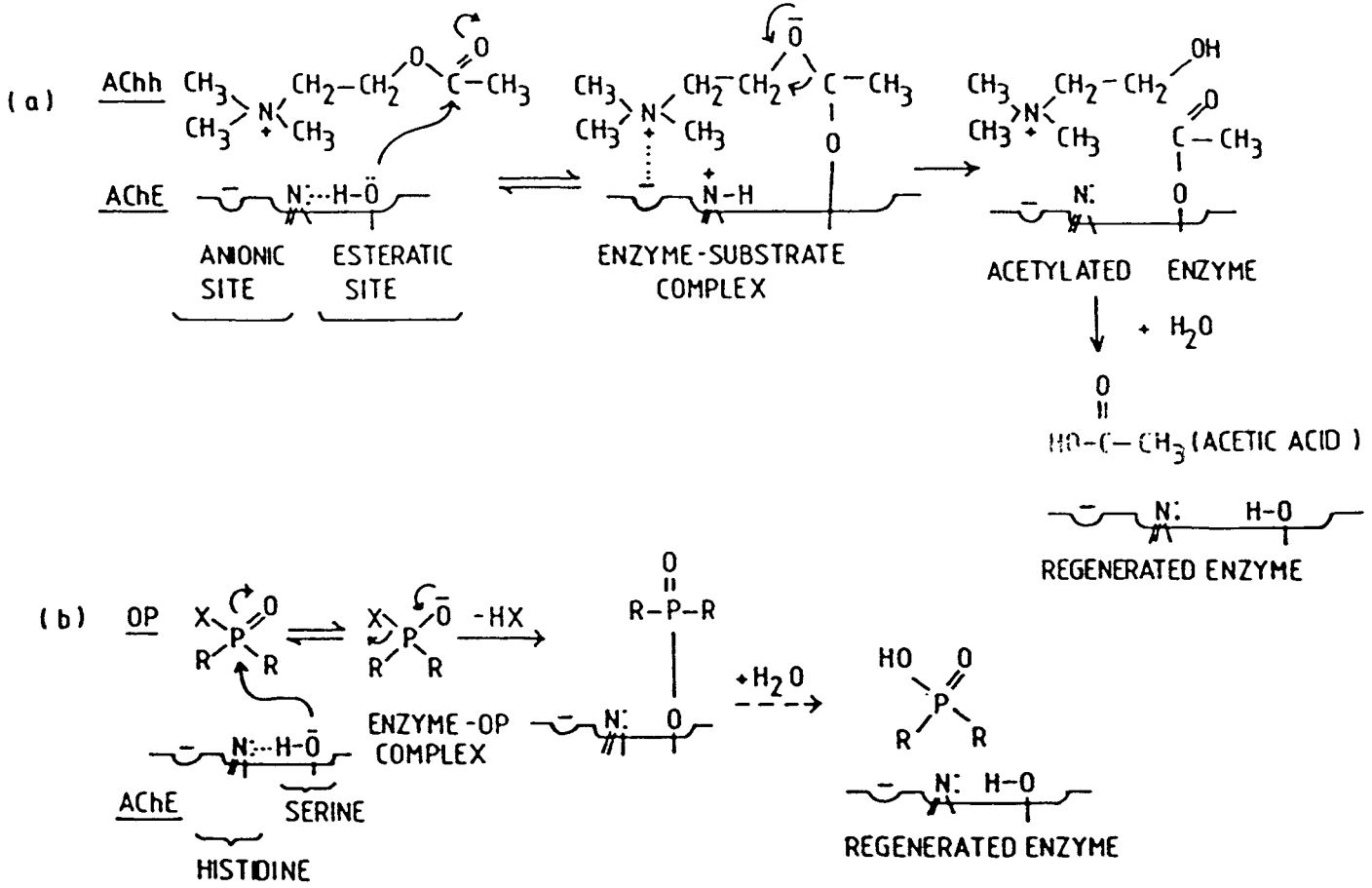


FIG.1-1: STEPS INVOLVED IN (a) HYDROLYSIS OF ACh BY AChE
(b) INHIBITION OF AChE BY ORGANOPHOSPHATE (OP).

several hours. Some OP compounds (e.g. ecothiophates), however, combine at both the esteratic and anionic sites, which probably contributes to their extreme potency and specifity.

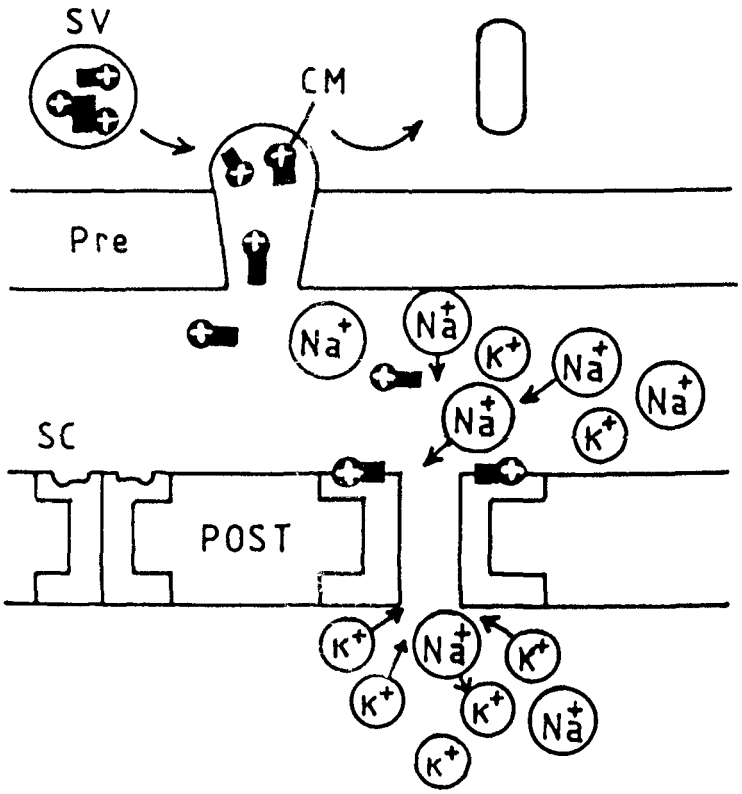
1.1.6 Mechanism of Nerve Transmission

The central nervous system (CNS) comprises of brain and spinal cord. The modes of communication between CNS and the rest of the body are through the peripheral nervous system. The communication channel is through cable lines of axons derived up from many neurons. Neuron, the basic structural unit of the nervous system, comprises the nerve cell body together with all its processes. The simplest neural action in vertebrates involves the participation of several neurons which form a common functional pathway. This functional conjugation is established via the synapses, consisting of a pre-synaptic membrane (usually an axon, rarely a dendrite) and a post-synaptic membrane (cell body, a dendrite or occasionally an axon), 20-30 nm apart.

The transmission of nerve impulse is an electrochemical process in which monovalent ions act as carriers of the current (Fig 1.2). Propagation of nerve impulse and the perturbation in the permeability of the axon membrane occurs simultaneously. The interior of the axon has larger amount of potassium (K) than sodium (Na) ions, while the fluid outside possesses an opposite composition. When a nerve impulse is generated, a flood-gate opens and the sodium ions rush into

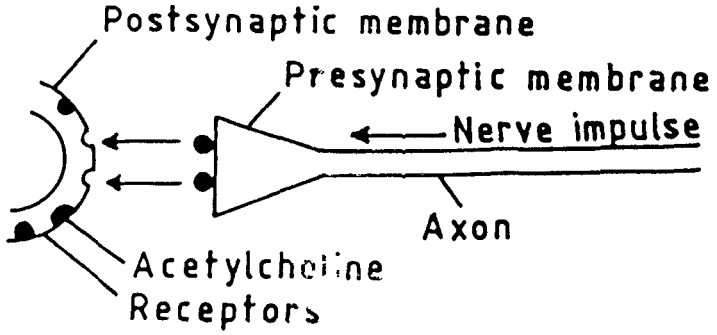
the axon, making the interior mileu locally positive. After the passage of the nerve impulse, the sodium gate closes and opens a potassium gate allowing for the efflux of K^+ ions and restoring the normal negative potential (Castillodel & Kotz 1957; Eccles 1957). The chemical transmitters carry the electrical potential across the gap between the pre-synaptic and post-synaptic membranes, called as "synaptic junction". The arrival of the nerve impulse at pre-synaptic membrane triggers the release of neurotransmitter, acetylcholine, from the 'vesicles' or storage sites located in pre-synaptic cells. ACh gets attached to a 'binding site' on the post-synaptic membrane after passing through the synaptic cleft (Fig 1.3). The neuromuscular junctions of the sympathetic system are stimulated by adrenalin or noradrenalin. It is however, possible that the adrenergic mechanism, too is set into motion primarily by ACh. In order to bring the ACh-induced action potential back to the resting state, and therefore facilitating the transmission of a new impulse, the liberated ACh is immediately broken down to acetic acid and inactive choline by the hydrolytic enzyme, AChE. A second enzyme, choline-o-acetyltransferase (Florkin & Stolz 1964) is capable of esterifying both compounds to acetylcholine again.

The inhibition of AChE leads to the accumulation of ACh at the synapses, thus preventing smooth transmission of nerve impulses across the synaptic cleft (Jonson 1976).



Diagrammatic representation of the basic principle of nerve impulse transmission.

Fig. 2·2



Ion fluxes and membrane involvement in nerve impulse transmission.

Fig. 2·3

1.1.7 Organophosphate Poisoning

The ever-increasing and indiscriminate use of organophosphates as pesticides, besides improving the agricultural yield, has been found to produce various toxicities in man and other non-target organisms (Hall & Kolbe 1980, Hasan and Ali 1981, Islam et al 1983, Tayyaba & Hasan 1985, Naqvi et al 1988, Khan and Hasan 1988, Naqvi & Hasan 1990, Katoh et al 1990, Tracey and Gallagher 1990, Naqvi and Hasan 1991 a,b&c).

Man is generally exposed to OP compounds either by consuming food grains, fruits and water contaminated with OPs, or while manufacturing, testing or spraying OP pesticides in the agricultural fields.

Some usual signs and symptoms of organophosphate poisoning in mammals are defaecation, urination, lacrimation, muscular twitching, fasciculations and convulsions, etc. (O'Brien 1960). The broad spectrum of OP-induced effects of the CNS include confusion, ataxia, slurred speech, loss of reflexes, generalised convulsions, central respiratory paralysis and coma.

The time of death following single acute exposure may range from less than five minutes to nearly twentyfour hours. The mechanisms involved in the death have been described by O'Brien (1960) as bronchoconstriction, lowered blood pressure, neuromuscular blockage of respiratory muscles and

failure of respiratory system. The sequence of events is as follows

- inhibition of ChE
- ACh accumulation at the synapses
- Disruption of nerve impulse
- Respiratory failure, and
- Death

1.1.8 Reports available on OP poisoning:

Numerous cases of OP poisoning in man have been reported from various parts of the world. The greatest of which was reported from Japan where 19, 436 cases of OP poisoning were registered during 1954-1970 (Ministry of Health & Welfare, Japanese Government 1954-1970). In Denmark 273 deaths were reported in six years. About 286 cases of OP poisoning occurred in Finland in six years (Toivonen et al 1959). Around 1950 deaths were registered in California in four years (Department of Public Health, State of California, 1959-1960). A study in Brazil revealed 3,445 cases of poisoning with 208 deaths during 1957-1979. In Mexico, four deaths were reported among 847 cases of poisoning in the agricultural workers in 1974. Twelve deaths were registered in 1987 in the agricultural workers of Punjab (Indian Express, July 22 1987).

Quite a large number of other fatal cases of organophosphate poisoning have been reported from all over

the globe (Vercruysse & Deslypere 1964; Gitelson et al 1965; Francis et al 1982; Vasilescue et al 1984, Tracey & Gallagher 1990).

In addition to these cases of accidental intoxication which resulted from use and manufacture of OP pesticides, these compounds have also been used quite often for homicidal and suicidal purposes. A study revealed that about 50-80% of poisons detected in 1969 from medicolegal cases in India belonged to organophosphate and organochlorine group of pesticides (Bami 1971).

1.2.0 Dimecron

"Dimecron", commonly known as phosphamidon, is a potent and commonly used systemic organophosphate insecticide belonging to the group of enol phosphates, also known as vinyl phosphates.

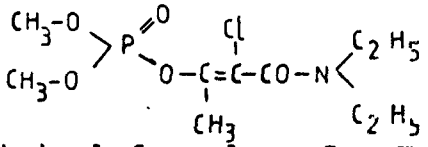
Phosphamidon was first synthesized in 1955 by E. Beriger, working in the laboratories of CIBA Ltd, Basle. The first screening trials revealed its most important features, systemic insecticidal activity; Low phytotoxicity; solubility in water; and lack of smell. Since it possessed these properties, phosphamidon was recognized as being particularly suitable for use as a plant protection agent. It has since become familiar under the name 'Dimecron' following several years of practical application in many countries.

1.2.1 Physico-chemical properties

Common name : Phosphamidon

Chemical name : 2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate

Structural formula :



Empirical formula: $C_{10}\ H_{19}\ O_5\ N\ Cl\ P$

Molecular weight : 299.7

Specific gravity : $d^{25}_4 = 1.2132$

Refractive index : $n^{25}_4 = 1.4718$

Boiling point : 0.04 mm Hg 94°C

0.2 mm Hg 115°C

1 mm Hg 150°C

Vapour pressure : 20°C $2.5.10^{-5}$ mm Hg

30°C $8.4\ 10^{-5}$ mm Hg

40°C $2.6.10^{-4}$ mm Hg

Volatility : 20°C 0.41 mg/m^3

30°C 1.33 mg/m^3

40° C 4.00 mg/m^3

Solubility: miscible with water and all organic solvents except saturated hydrocarbons, in which it is soluble only to a limited extent.

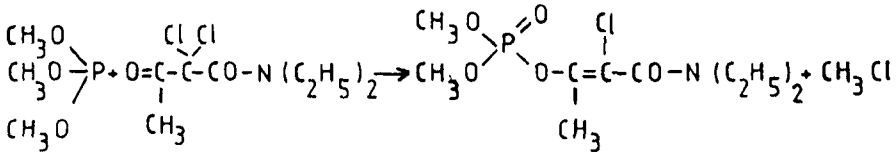
Viscosity : 25° = 70 Cp.

Colour : Colourless to pale yellow (commercial form is coloured bright violet by the addition of dye for safety purposes)

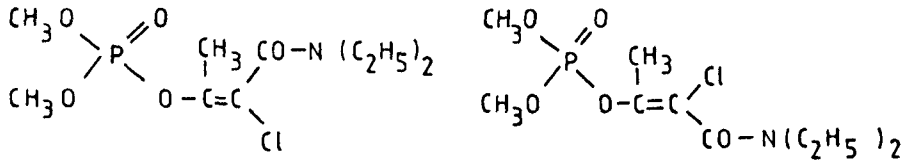
Odour : faint, pleasant

1.2.2 Synthesis, isomers

Phosphamidon is synthesized by condensing -halocarbonyl compounds with trialkyl phosphites, according to the Perkow reaction (Lichtentahler 1961). The general equation can be represented as follows:



This reaction gives two isomers, the -isomer (cis-phosphamidon) and -isomer (trans-phosphamidon), which differ in the arrangement of the substituents at the C=C double bond:

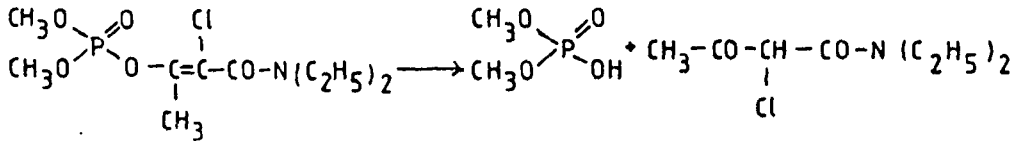


These two isomers differ as regards biological activity. The insecticidal activity of the -isomer which accounts for 70% of technical grade phosphamidon, is several times greater than that of the -isomer. Similarly -isomer possesses more potent anticholinesterase activity.

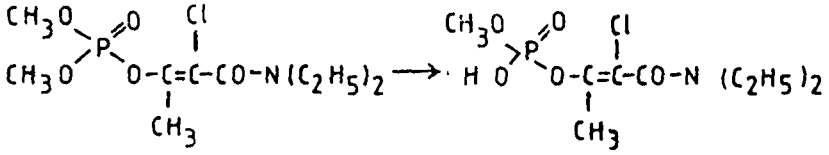
1.2.3. Metabolism

Various studies have revealed three ways in which phosphamidon is broken down in biological substrates (Anliker et al 1961). By the first process, phosphamidon is hydrolysed to give dimethyl phosphoric acid and N, N-

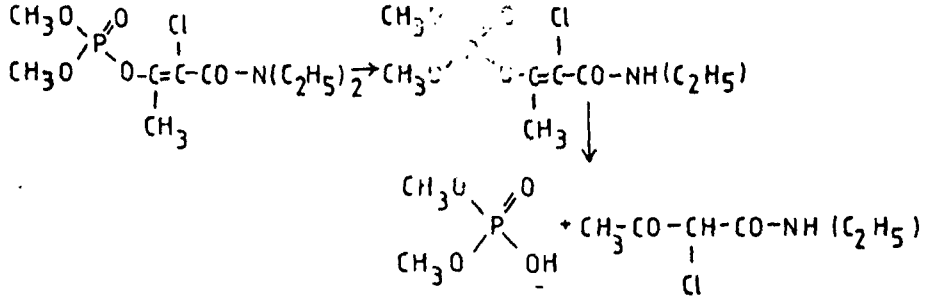
diethylchloroacetoacetamide; the latter is then decomposed in an acid environment to give chloroacetone, carbon dioxide, and diethylamine. In the alkaline environment, first of all hydrochloric acid is formed and then, considerably more slowly, acetic acid, the final product being glycolic acid diethylamide.



By the second process, the phosphate group is demethylated to give desmethylphosphamidon; this process can, in theory, also be regarded as hydrolysis, but in practice is probably more complicated:



The third process consists of the removal of one ethyl group from the acid amide. The resultant desethyl phosphamidon is further broken down to give N-ethylchloroacetoacetamide, which is again decomposed into smaller compounds:



1.2.4 Toxicology

Dimecron, being an organophosphate pesticide, as such possesses the biological property of inhibiting the enzyme acetylcholinesterase (AChE) (Klotzshe 1958, Jaques & Bein 1960, CIBA monograph 1967). Various neurochemical and neurobehavioural effects of dimecron have been reported (Gitelson et al 1965, Naqvi et al 1988, Naqvi & Hasan 1990 1991a,b,c).

The no-effect level of dietary intake of pesticides established for the most sensitive animal species is taken as a basis for calculating an "acceptable daily intake" (ADI) for man. It is defined as "the daily dosage (expressed in mg of the chemical present in the food per Kilogram body weight) which during an entire life time appears to be without appreciable risk on the basis of all the facts known at that time". This definition was formulated by the Food and Agriculture Organization (FAO) committee on pesticides in agriculture, and the WHO expert committee on pesticide residues (FAO/WHO 1964). Toxicological investigations have shown that in the rat and dog, the no-effect level of dimecron as regards growth rate, pathological and histopathological findings, blood and renal and liver function, is of the order of 2.5 to 5 mg in the food Kg^{-1} / day. Dividing this by the safety factor of 100 gives an ADI for man as 0.025 mg / Kg (CIBA monograph 1967). On the other hand, the cholines. . se no-effect level of 0.1 to 0.5

mg / Kg / day, divided by the safety factor 10, gives an ADI for man of 0.01 mg / Kg, equivalent to the daily ingestion of 0.5 mg by an adult weighing 50 Kg.

Interestingly, Naqvi and Hasan (1990) have reported dose-related accumulation of dimecron and its metabolite desethylphosphamidon in the CNS regions of rats treated with graded doses (1.0, 1.5 and 2.0 mg Kg^{-1} b.wt, ip) of dimecron for seven days.

1.2.5 Signs and symptoms:

Phosphamidon has been found to induce the signs and symptoms of poisoning similar to those produced by most of the other anticholinesterase organophosphate compounds. A list of sings and symptoms of general anticholinesterase poisoning (Holmstedt 1959; WHO 1962) is given in Table 1.5.

TABLE 1.5

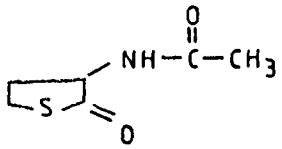
Site of action	Signs and symptoms
(a) Following local exposure	
- Pupils	: Miosis, marked, usually maximal, sometimes unequal.
- Ciliary body	: Frontal headache, eye pain on focussing, dimness of vision, nausea and vomiting.
- Nasal mucous membrane	: Rhinorrhoea, hyperaemia.
- Bronchial tree	: Tightness in chest, bronchoconstriction, cough.

Table 1.5 contd...

- Sweat glands	:	Sweating at site of exposure.
- Striated muscles	:	Fasciculations at site of exposure
		(b) Following systemic absorption:
- Bronchial tree	:	Tightness in chest, bronchoconstriction, cough, slight pain in chest, increased bronchial secretion.
- Gastrointestinal system	:	Anorexia, nausea, vomiting, abdominal cramps, epigastric and substernal tightness, diarrhoea, involuntary defecation.
- Sweat glands	:	Increased sweating.
- Salivary glands	:	Increased salivation.
- Lachrymal glands	:	Increased lachrymation.
- Heart	:	Slight bradycardia.
- Pupils	:	Slight miosis
- Ciliary body	:	blurring of vision
- Bladder	:	Frequent or involuntary micturition
- Striated muscle	:	Easy fatigue, muscular twitching, fasciculations
- Central nervous system	:	Giddiness, tension, anxiety, restlessness, excessive dreaming, headache, tremor, drowsiness, confusion, slurred speech, ataxia, convulsions, coma, depression of respiratory and circulatory centres, fall in blood pressure.

1.2.6 Treatment:

Phosphamidon poisoning can readily be antagonised by the use of known antidotes of organophosphorus cholinesterase inhibitors, such as atropine and PAM (2-pyridine aldoxime methyl iodide). Furthermore, an antioxidant agent, acetylhomocysteine thiolactone, commonly known as cithiolone, has also been reported to protect against experimental phosphamidon toxicosis (Hasan et al. 1987, Naqvi and Hasan 1991a).



Structure of Cithiolone

1.3.0 Open field Behaviour (OFB)

It is well known that changes in the biochemical mechanism and amine concentrations in the CNS may lead to varying disorders in behaviour (Taylor and Snyder 1971). A number of CNS active drugs and chemicals have been found to alter the open field behaviour on acute poisoning. Depending upon the site and mechanism of action, various OFB parameters i.e. ambulation, rearing and preening show varying disorders.

Ambulation is described as horizontal (simple) stereotypy (Dandiya et al 1969). It is indicative of intact muscular coordination. Rearing is a vertical (complex) stereotypy, and dopamine plays an important role in its

control (Gupta and Holland 1972). It is indicative of cortical stimulation (Lat 1965). Preening is a behavioral response and has been said to be a behavioural correlate of cortical stimulation (Gupta et al 1971).

Ali et al (1980) have correlated varying perturbations in the open field behaviour with altered monoamine levels in the brain following organophosphate dichlorvos toxicosis.

1.4.0 Acetylcholinesterase (AChE)

Acetylcholinesterase is manufactured in cholinergic cell bodies. It is delivered to the synapses by axonal transport. It is the most important cholinesterase enzyme, which hydrolyses the cholinergic neurotransmitter, acetylcholine (Ach) at the synapses after transduction of nerve impulses, thereby, paving the way for the smooth transmission of nerve impulses. Koelle (1963) noted that the only part of the AChE of brain behaves as if it were accessible to quaternary substrates and inhibitors. He supposed that this fraction of the enzyme (functional AChE) is outward facing, and the remainder (reserve AChE) is inward facing and in transit. The ability of peripheral cholinergically innervated tissue to form surplus ACh (Collier & Katz 1971) in the presence of an anticholinesterare suggests that at least a small part of the enzyme transported by peripheral axons is still in the reserve orientation as it nears the synapses, cerebral

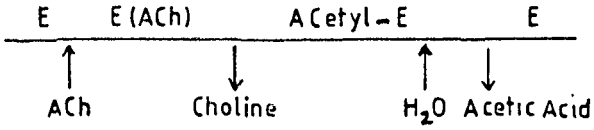
cortex, which seems to have less of that ability, may possess only functional AChE.

Most tissues contain several forms of AChE (Haubrich & Chippendale 1977; Bon et al 1979). These are catalytically identical glyerproteins, but they differ in molecular weight, ease of extraction and physical properties. In order of increasing size, they are a monomer of about 65,000 daltons, the likely precursor of other forms; a dimer and a tetramer, which are partly in solution and partly membrane bound; and several asymmetric species that have a cluster of monomers attached to a collagen tail. In neurons, the enzyme is transported along the axon and eventually externalized; when this happens part remains attached to the neurilemma, but part is secreted into the extracellular space (Skau & Brimijoin 1978). At nicotinic junctions the collagen-tailed variants predominate; they are not membrane-bound, but reside in the synaptic cleft.

The active center of each enzyme subunit has been described as incorporating "anionic" and "esteratic" sites which fit respectively the cationic trimethyl cluster and the ester carbonyl of ACh, but binding at the anionic site depends more on hydrophobic than on coulombic reaction. The esteratic site incorporates a serine side-chain, like many hydrolytic enzymes; a histidine imidazole is also present.

The irreversible (or very slowly reversible) organophosphates, including important pesticides as well as

nerve gases, inactivate the AChE through binding themselves to the esteratic site of the enzyme, and thus phosphorylating the serine hydroxyl (Hall & Kolbe 1980). This leads to the accumulation of ACh at the synapses, causing disruption of smooth nervous transmission (Murphy 1980). A detailed mechanism of inhibition of AChE by organophosphates has been given in an earlier section (1.1.5). Kinetic studies of the enzyme indicate a three-step reaction with AChE (Fig 1.4).



The final deacetylation is probably the rate controlling step. AChE is one of the fastest enzymes, with a turnover number higher than 10^{-4} sec^{-1}; but its velocity falls off at high substrate concentrations, because of the increased probability that two ACh molecules might attach at a single active centre and thus prevent each other's hydrolysis (substrate inhibition).

Acetylcholine (ACh), unlike norepinephrine and other neurotransmitters, is not recaptured to any extent by the nerve endings that have released it, only the choline derived from its breakdown can be taken up and reused. So, AChE has a double function: on the postsynaptic site, it prevents released ACh from acting longer than it is necessary; and on the presynaptic site, it helps to ensure an adequate supply of choline for ACh synthesis.

A number of studies have revealed that most of the organophosphate pesticides inhibit the AChE activity in the brain. The variations in the extent of toxicity of various OP compounds are supposed to be due to variations in the rate of their enzymatic hydrolysis (March et al 1956). DuBois et al (1949) observed that rats poisoned with OP, parathion, showed reduced cholinesterase activity. Paul et al (1979) observed malathion-induced inhibition of AChE. Studies of Tsumuki et al (1970) reveal AChE inhibition by dimethoate, sumithion and trichlorfon, in varying proportions depending on the level of, and duration of the pesticide treatment. Diazinon has been reported to inhibit ChE activity in the CNS and other organs (Weiss et al 1964) and increase the level of ACh in the brain (Kar and Matin 1971), resulting in stimulatory effects, tremors and convulsions. Ali et al (1977) reported diminution of AChE in the rat brain following dichlorvos intoxication. Most of the earlier workers were of the view that OP-induced AChE inhibition is irreversible, but Aldridge (1953) reported a recovery in the AChE activity following its depletion by organophosphate triesters. The studies of Nagaratnamma & Ramamurthy (1983) in Cryrinus carpio with methyl parathion also indicate rapid recovery of brain AChE. Although abundant literature is available on the effects of various OPs on the AChE, the neurotoxic effect of dimecron on the brain AChE has not been reported so far.

1.5.0. Monoamines

Monoamines, consisting of catecholamines (CA) and serotonin (5-HT) act as important neurotransmitters in the brain. Localized in the nerve fibres and nerve terminals, they are universally distributed in the CNS, especially in the regions to which autonomic or vegetative functions are attributed (Friede 1966).

The catecholamines: dopamine, norepinephrine and epinephrine, are neurotransmitters, concerned with a number of functions related to emotion, attention and visceral regulation. Although dopamine (DA) serves as the precursor for norepinephrine (NE), and NE may be N-methylated to form epinephrine, the three monoamines have separate localizations in distinct neuronal pathways (Coyle & Snyder 1981). The cell bodies of dopaminergic neurons are located primarily in the mid-brain, while the noradrenergic neurons cluster in the medulla oblongata, pons and mid-brain, in particular locus coeruleus of the floor of fourth ventricle.

Catecholamines are concentrated within storage vesicles that are present in high density within the nerve terminals. Ordinarily low concentrations of the catecholamines remain free and unprotected in the cytosol. Thus, during biosynthesis, conversion of tyrosine to L-dopa and L-dopa to dopamine occurs in cytosol. Dopamine, then is taken up within the storage vesicles. For the noradrenergic neurons, the

final hydroxylation occurs within the vesicles. The mechanism that concentrates the catecholamines within the vesicles is an ATP-dependent process linked to a proton pump (Holz 1978). The vesicles play a dual role: they maintain a ready supply of catecholamines at the terminal, and they mediate the process of their release. When an action potential reaches the nerve terminal, calcium (Ca^{++}) channels open, allowing an influx of the cation into the terminal; increased intracellular Ca^{++} promotes the fusion of vesicles near the synaptic specialization with the neuronal membrane. The vesicles then discharge their soluble contents, including NE, AT and DBH, into the extraneuronal space (Coyle & Snyder 1981). Inspite of marked fluctuations in the activity of catecholaminergic neurons, the levels of catecholamines within the nerve terminals remain relatively constant. Through such mechanisms as end-product inhibition, transient activation, and enzyme induction, the neuron can accommodate to alterations in utilization of catecholamine neurotransmitters (Joh et al 1978). The action of catecholamines released at the synapse is terminated primarily by reuptake into the nerve terminals that release the neurotrasmitters. Catecholamines that have not been removed from the synaptic cleft by the transport process diffuse into intracellular space, where they are catebolized by the enzyme, monoamine oxidase (MAO) and cathecol-o-methyl transferase (COMT) (Fig 1.5). The catecholamine reuptake process was discovered by Axelrod (1971).

Serotonin or 5-hydroxy tryptamine (5-HT) is widely distributed in the body of coelenterates, arthopods, molluscs, tunicates and vertebrates. Like acetylcholine and catecholamines, serotonin acts on many body organs either directly or by a neuronal reflex. Its actions on the cardiovascular, respiratory and gastrointestinal systems are especially prominent.

The CNS contains a relatively small amount (about one percent) of the serotonin in the body. The distribution of serotonin in the brain is uneven. The brain-stem has the highest concentration, the cerebral cortex has moderate while cerebellum has a low concentration (Saavedra et al 1973). A family of neurons in the pons and mesencephalon is the primary source of serotonin in the brain (Michael 1981). These neurons are found in or near the midline, or in the raphe nuclei, in clusters. Sertonin is stored in synaptic vesicles in nerve endings. It appears to be synthesized outside the vesicles and then taken into them (Joh et al 1975). Finally, it is released by an exocytotic process. The most important enzyme in catabolising serotonin is monoamine oxidase A, which oxidizes the amino group of serotonin to form an aldehyde, 5-hydroxyindoleacetaldehyde, which usually oxidizes to yield 5-hydroxyindoleacetic acid (5 HIAA) (Fig. 1.6). Enzymatic degradation by MAO helps to limit the biological actions of 5-HT (serotonin), so does reuptake into nerve terminals. Both serotonergic and other monoaminergic

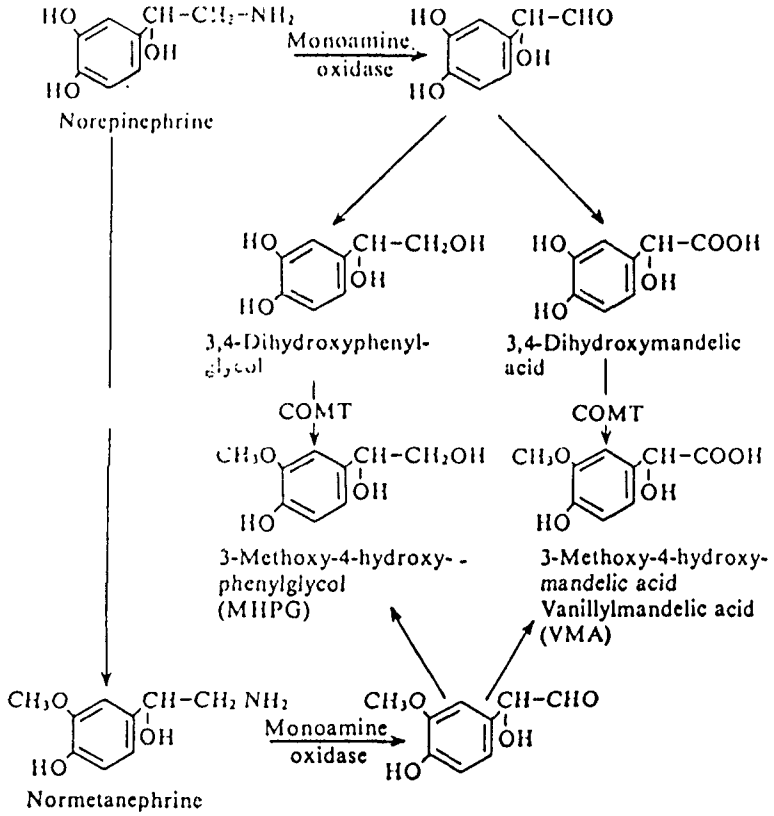


Fig. 1·5 : PATHWAYS OF NOREPINEPHRINE DEGRADATION.

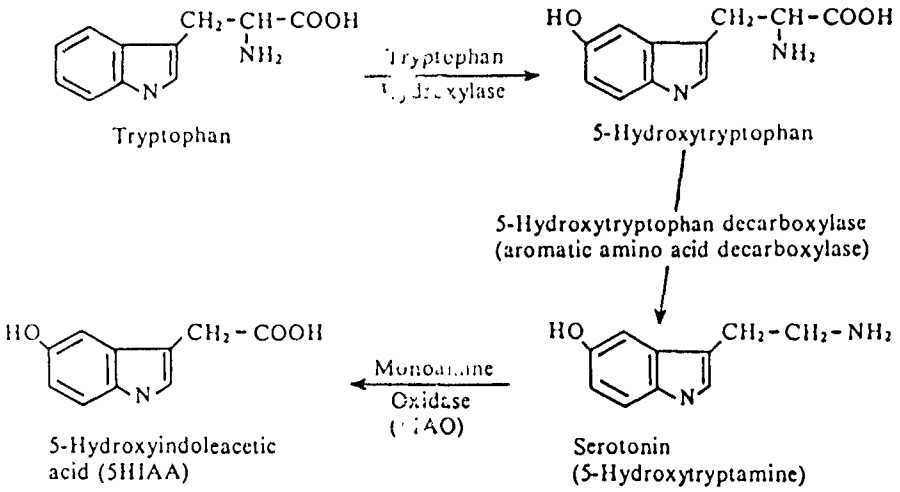


Fig. 1·6 : METABOLISM OF SEROTONIN.

terminals can take up [illegible]. Occupation of presynaptic 5-HT "autoreceptors" on serotonin-containing neurons reduces the neuron's rate of spontaneous firing . This provides a mechanism for feedback inhibition of neuronal activity that is coupled to the release of transmitter.

5-HT has been implicated in numerous central processes, including regulation of the anterior pituitary, sleep, perception of pain, thermoregulation, control of blood pressure, appetitive behaviour, drinking, respiration, heart-rate, rhythmic behaviour, and behaviour.

Some of the organophosphates e.g. parathion (Fiscus & Van Meeter 1977) and dichlorvos (Ali et al 1980) have been reported to purterb the levels of monoamines in the brain. However, no report is available todate on the effect of OP dimecron on the monoamines.

1.6.0 Monoamine Oxidase:

Monoamine oxidase (MAO) is an important neuronal enzyme that is primarily responsible for the catabolic inactivation of monoamines. It is a flavin-containing enzyme located on the outer membrane of the mitochondria (Costa & Sandler 1972). MAO catabolically degrades monoamines by oxidatively deaminating them to their corresponding aldehydes, in turn, these can be converted to analogous acids by aldehyde dehydrogenase (Cooper et al 1978). Role of MAO in the catabolism of monoamines is depicted in Figures 1.5 and 1.6

MAO catalyzes the following reaction:

$$RCH_2CH_2NH_2 + O_2 + H_2O \longrightarrow RCH_2CHO + H_2O_2 + NH_3$$

Because of its intracellular localization, MAO plays a strategic role in inactivating catecholamines that are free within the nerve terminal and not protected by the storage vesicles (Coyle & Snyder 1981). Isoenzymes of MAO have been characterized with differential substrate specificities; MAO-A preferentially deaminates NE and 5-HT, whereas MAO-B acts on a broad spectrum of phenyl ethylamines.

It is well known that biogenic amines are involved in the regulation of certain activities of the central nervous system (Chase & Murphy 1973). Any change in the normal amine concentration will disturb their activities and result in convulsive seizures (Killen & Frey 1973). Organophosphate pesticides have been reported to deplete the brain monoamine levels (Ali et al 1980; Drezezinsky & Parus zewska 1980; Naqvi & Hasan 1991c). Also, OPs have been found to be MAO activators (Ravi 1984, Naqvi & Hasan 1991b). When treated with a MAO activator, the synthesis of monoamine is essentially unaffected but due to enhancement in the degradation of amines, a net decrease in the amine concentration is observed (Sourkes 1981).

1.7.0 Brain Lipids

Among various body organs, the brain is one of the richest in lipids. Studies of lipids in the nervous system, are therefore, a vital part of the neurochemical investigation due to the immense importance of lipids in the brain, both as structural constituents and as participants in the functional activity of the brain. Lipids account for about half the dry weight and most of the structural architecture of membranes in the brain (Brante 1949; Ordy & Kaack 1975, Suzuki 1981). Brain contains a unique structure, the myelin sheath, which has the highest lipid concentration of any normal tissue or subcellular components, except for adipose tissue. Total lipids make up about 65% of the dry weight of white matter and 35 to 40% of the gray matter (Brante 1949). Myelin sheath contains about 65% of the lipids of whole white matter. Myelin is present in all parts of the CNS, but is more concentrated in areas composed mainly of fiber tracts, such as the white matter of brain and spinal cord and in peripheral nerves, such as sciatic nerve. Different types of membranes accumulate different types of lipids (Horrocks et al 1975). There is increasing evidence that lipid molecules play important functional roles within the membranes. Some of the postulated physiological functions of membrane lipids include the site for the cell-to-cell recognition process, specific cell-surface antigens, and

specific receptors for toxins or other physiological compounds.

The lipid composition of the brain is relatively stable throughout adult life. However substantial changes in lipid composition take place during active myelination. In active myelination, the brain loses water, predominately in white matter, the lipid content increases rapidly and the differences between gray and white matter become more apparent (Wells & Dittmer 1967; Norton & Poduslo 1973). The rapidly increased myelin content is related with increase in brain weight (Smith et al 1983). Cumings (1955) compared the lipids of demyelinated lesions in multiple sclerosis with those of normal areas of the brain and found a decrease in sphingomyelin, cerebroside and free cholesterol. Phosphoglycerides, cholesterol and galactolipids are present in mature myelin sheath, with the mole ratio of 3:4:2 respectively. Gangliosides are localized primarily in plasma membranes (Karpova 1978) but monosialoganglioside is present in limited quantity in myelin fractions (White et al 1978).

Most of the membrane lipids exist in a "bilayer form" according to 'Fluid Mosaic" model of Singer and Nicolson (1972). The physical state of the lipid plays a critical role in directing the conformation and function of the membranes. The lipid bilayer of the membranes is predominantly composed

of phospholipids and variable amounts of cholesterol and glycolipids (Tanford 1978).

There is good deal of experimental evidence to show that organophosphates influence lipid synthesis in the brain (Majno & Karnovsky 1955). Organophosphates not only influence the lipid metabolism, but also alter the lipase activity (Caley and Jenson 1973; Islam et al 1983). Nelson and Barnum (1960) observed the inhibition of phospholipid biosynthesis after toxicosis with OP pesticide, di-isopropyl phosphorofluoride. Previous studies from this laboratory have revealed perturbations in different brain lipid levels following exposure to various OP pesticides, viz. dichlorvos, metasystox, methyl parathion (Tayyaba & Hasan 1980; Islam et al 1983; Tayyaba & Hasan 1985; Vadhwa & Hasan 1986, Hasan & Khan 1985; Khan & Hasan 1988). In the present study, effect of OP, dimecron on the lipid levels in the CNS have been investigated because, to date, no report is available on the neurotoxic effects of dimecron on brain lipids.

1.7.1 Phospholipids

Phospholipids are the primary lipid components of plasma membranes, organic membranes and myelin membranes. The importance of these phosphorus containing lipids in the CNS presumably depends upon their role as membrane constituents. Phospholipids constitute approximately one-fourth of the dry weight of brain (White 1973). They account for 4.1% of fresh

weight and 22.7% of dry weight of the gray matter, and 7.2% of fresh weight and 25.2% of dry weight of the white matter (Suzuki 1981). The lipids of mammalian CNS myelin are composed of 4.6% phospholipids and hence it is proposed that phospholipids are the characteristic components of myelin sheath (Fumagalli & Paoletti 1963). The metabolically active polyphosphoinositides are located in the excitable membrane of the neuron, particularly the axolemma. Phospholipids not only constitute the backbone of biomembranes, but also provide the membrane with a suitable environment, fluidity and ion permeability. Porcellati (1983) reported that different phospholipids turnover at different rates with respect to their structure and localization in various cells and membranes. Newly synthesized phospholipds are transported to membranous structures by phospholipid exchange and transfer proteins (Demel et al 1984) which are found in the cytosol. The distribution of phospholipids in biological membranes is thus regulated not only by the activities of enzymes involved in their metabolism but also by the transport and incorporation processes into the membrane. Metabolic pathways of phospholipids in brain are similar to those in systemic organs (Rossiter 1966) and are depicted in Fig.1.7.

Several organophosphates have been reported to deplete the level of phospholipids in the CNS (Nelson and Barnum 1960; Tayyaba & Hasan 1985; Hasan & Khan 1985; Vadhva & Hasan

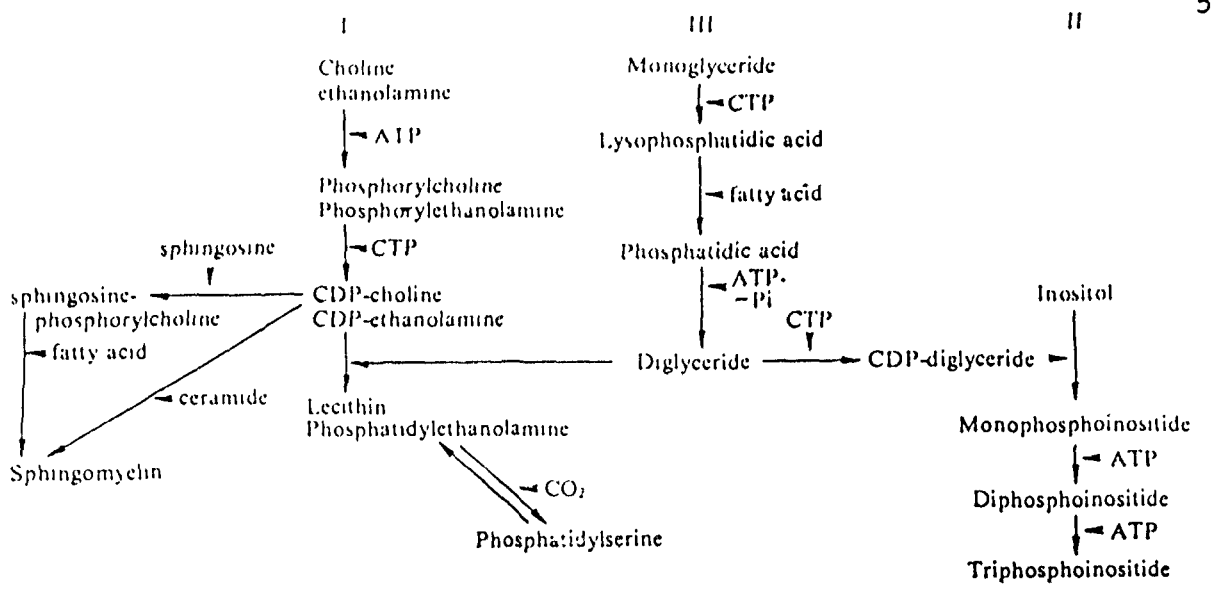


Fig.1·7: METABOLIC PATHWAYS OF PHOSPHOLIPIDS.

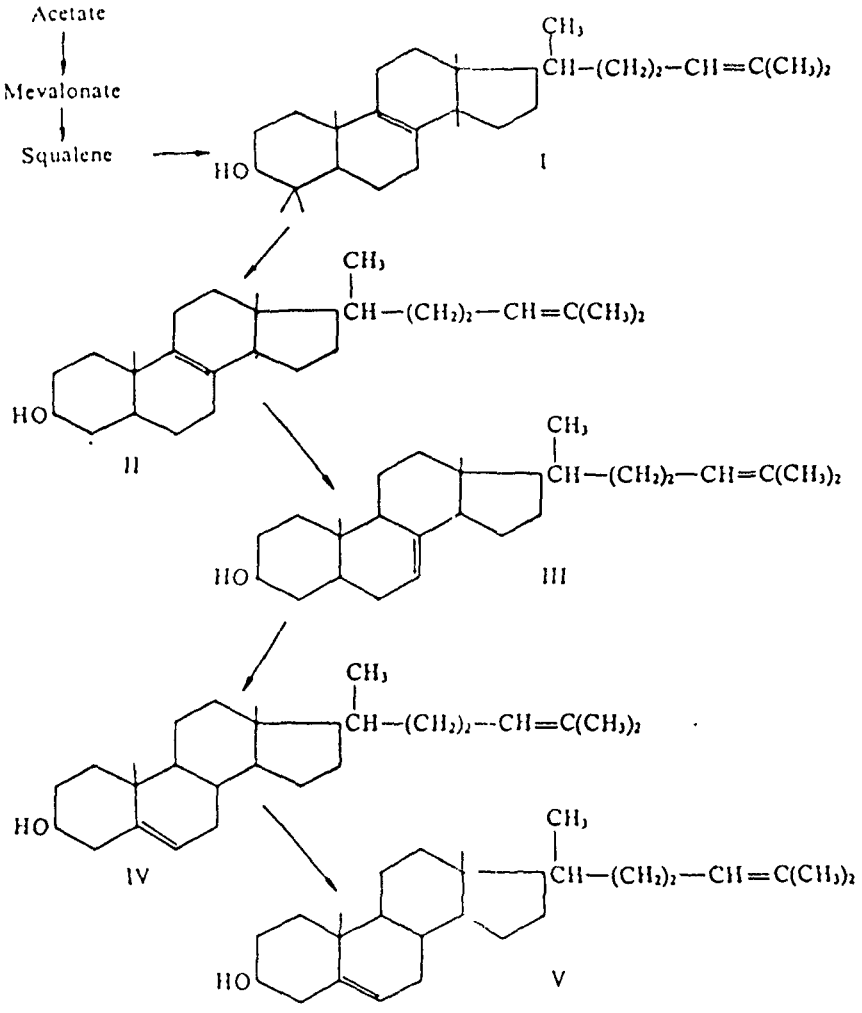


Fig.1·8: OUTLINE OF CHOLESTEROL BIOSYNTHESIS.

1986, Naqvi et al 1988). As per the available literature, however, dimecron has not been much studied regarding its neurochemical effect on the phospholipids.

1.7.2 Cholesterol

Widely distributed in other body organs, cholesterol is found in highest concentration within the central and peripheral nervous systems. Cholesterol accounts for about 10% of dry weight of the brain as compared to less than 1% found in most of the other organs. This high cholesterol level seems to be characteristic of nervous tissue (Ansell & Hawthorne 1964). Unesterified cholesterol has been suggested as a lipid characteristic of myelin sheath, as it occurs in white matter in a higher concentration than in gray matter (Johnson 1949; Brante 1949). Cholesterol constitutes approximately 25% of myelin lipid by weight (Soto et al 1966) and about 70% of total brain cholesterol is present in myelin (Laatsch et al 1962). Cholesterol is thought to act as a conveyer in the absorption of fats. Bloor (1943) reported a parallelism between cholesterol content of blood and the fatty acids. Due to the abundance of cholesterol in nervous tissue and its variation in mental diseases, it may function as an insulating medium for myelin sheaths. Adams (1961) indicated a strong reaction in the myelin sheath of peripheral nerve and fibre tracts of the brain. Sterols are thought to have a role in maintaining the balance between the cell permeability and the membrane equilibrium of living

cells. Paoletti (1971) has described microsomes as the subcellular site of cholesterol biosynthesis in brain. Cholesterol biosynthesis takes place in the brain through the same pathway as in systemic organs (Suzuki 1981). Acetate and its precursors are transformed through mevalonic acid to cholesterol (Fig.1.8). Desmosterol, the immediate precursor of cholesterol, is known to be present in brain in measurable amounts just prior to myelination (Paoletti et al 1965) and also in the myelin sheath itself in the early stage of myelination (Smith et al 1967) Biosynthesis of cholesterol in brain is most rapid during active myelination, but adult brain retains the capacity to synthesize cholesterol when precursors, such as acetate or mevalonate are available.

Previous studies indicate that certain organophosphate pesticides perturb the cholesterol contents in various CNS regions (Tayyaba & Hasan 1980; Islam et al 1983; Nag & Ghosh 1984; Rao & Rao 1984; Tayyaba & Hasan 1985; Hasan & Khan 1985; Vadhva & Hasan 1986; Naqvi et al 1988). To our knowledge, to date, no attempt has been made to study the neurotoxic effects of dimecron on the cholesterol levels.

1.7.3 Gangliosides

Gangliosides are defined as sialic acid-containing sphingoglycolipids. Sialic acid is the generic name for N-acylneuraminic acid, and the acyl group of sialic acid in the human brain is always the acetyl form. (Suzuki 1981). N-acetylneuraminic acid is commonly abbreviated as New NAc

(Fig.1.9). The series of gangliosides in the brain has the ceramide rligohexosides as the backbone, with one or more Neu- NAc moieties attached. Major gangliosides of the brain are GM1, GM2, GM3, GD1a, GD1b GT1. Several other minor gangliosides have been identified in the nervous system, including GD_3, GD_2, and sialylgalactosylceramide. Gangliosides are the essential constituents of the cell membrane. They are more concentrated in the CNS than in other body organs (Svennerholm 1980, Rahman 1983). In the gray matter, 6% of the total lipids are gangliosides (Lehninger 1984). Also, gray matter contains many times higher ganglioside concentration, compared to white matter (Wolfe 1972). Moreover, at the cellular level in the brain, gangliosides are more highly concentrated in the neurons than in the glial cells and myelin (Roberts 1975, Ledeen 1978, Yu & Iqbal 1979). Regional differences in ganglioside patterns of the nervous system have also been recognized. In adult nervous system, the individual gangliosides have been suggested to play a role as membrane-bound receptors or co-receptors for toxins, drugs, viruses, hormones, transmitters etc. (Svennerholm 1980). Although no convincing evidence is available linking gangliosides to specific neuronal function, they may be involved in synaptic transmission (Rahman et al 1982), owing to their binding capacity for calcium (Probst et al 1979) and transmitters (Richardson et al 1982).

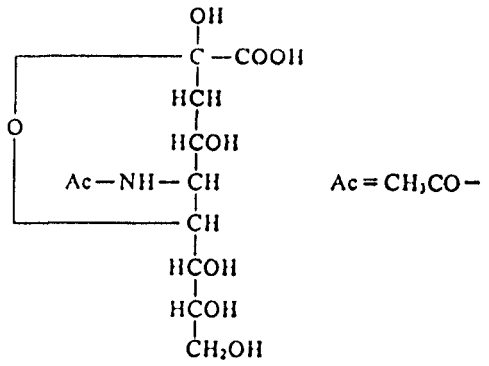


Fig.1·9 : STRUCTURE OF ACETYLNEURAMINIC ACID

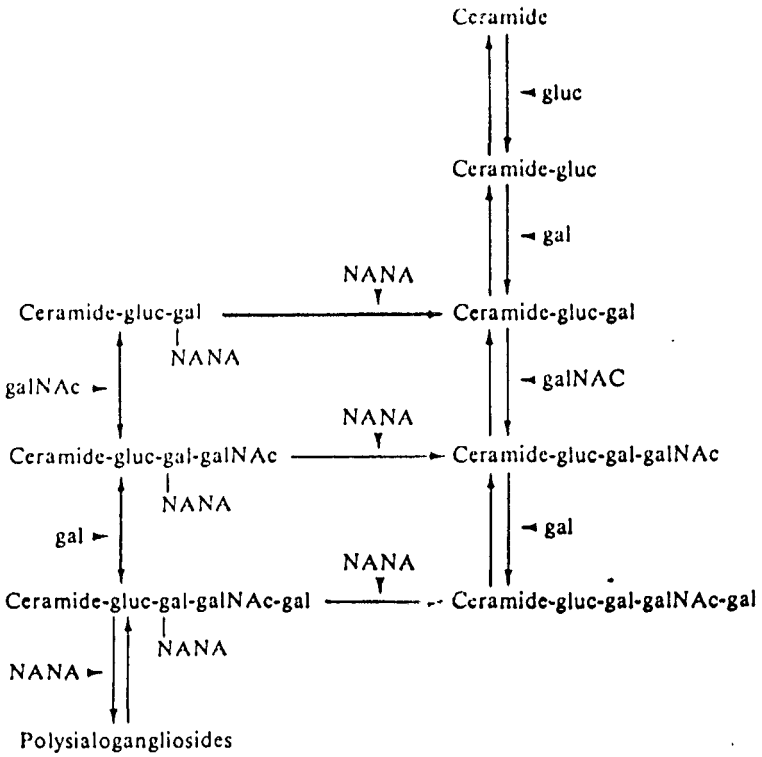


Fig.1·10 : OUTLINE OF GANGLIOSIDE METABOLISM.

Brain Gangliosides are biosynthesized by sequential additions of monosacchardies or Neu NAc to the carbohydrate chain, starting from ceramide (Fig.1.10) (Kaufman et al 1967, Ledeen & Mellanby 1977). The biosynthetic steps are catalysed by either sialyl or glycosyl transferases that are generally specific for the respective reactions. Although the bulk of the synthetic activities are localized in the microsomal fraction, there is evidence to suggest that some ganglioside sialyltransferase may also be present in nerve endings (Suzuki 1981). Degradation of brain gangliosides also proceeds by sequential removal of monosaccharide and Ncu NAc (Fig.1.10) by glycosidases and neuraminidases (Ladeen & Yu 1973); Ledeen and Mellanby 1977). Most of these hydrolytic enzymes are localized in lysosomes, wherein the catabolic processes of cellular constituents are believed to occur (Sandhoff & Christmanou, 1979).

Because of the high concentration in the neuronal membrane (Ledeen 1978) and the unusual chemical structure of both hydrophilic and hydrophobic chains, the physiological functions of brain gangliosides, such as their static role as membrane constituents, and their possible involvement in ion transport and nerve transmission, have been the subject of speculation. GM_1 can affect neurotransmitter receptors with a modulatory action observed on cortical 5-HT receptors (Agnati et al 1983). Gangliosides have also been shown to modulate

the phosphorylation system (Agnati et al 1984 1985; Bremer et al 1986).

Previous reports reveal that some organophosphate pesticides have been found to deplete the ganglioside concentrations in various CNS regions (Islam et al 1983, Tayyaba & Hasan 1985, Naqvi et al 1988, Khan & Hasan 1988), but the effects of dimecron have to date received little attention.

1.8.0 Lipid Peroxidation:

A recently applied approach to the study of neurotoxicology involves the investigation of lipid peroxidation (WHO 1986). The lipids within the membranes of cells from higher organisms contain large numbers of polyunsaturated fatty acid side-chains. Such fatty acids are prone to undergo a process known as "lipid peroxidation'. It is a basic detereorative reaction that is involved in many disease processes and chemical toxicities (Tappel and Dillard 1981). Recent interest in lipid peroxidation has probably resulted from the realization that oxygen radicals do exist in biological tissues for an appreciable time (Tien et al 1981). Lipid peroxidation is a process of direct reaction of oxygen with lipid to form free radical intermediates and hence creating semi-stable peroxides. Because of their unpaired electrons, free radicals react energetically and initiate relatively non-specific hydrogen abstraction and

chemical addition reactions. Major sites of lipid peroxidation are biomembranes and subcellular organelles (Tappel 1970). Lipid peroxidation "in vivo" has been found to be of basic importance in ageing, damage to cells by air pollution, toxic chemicals and in oxygen toxicity (Tappel 1970). It has been found that loss of membrane integrity during pathological free radical mechanism leading to lipid peroxidation and degeneration of phospholipids are important factors which irreversibly damage brain cells in ischaemic and other adverse conditions (Demopoulos et al 1979). The peroxidation of lipids can be highly catastrophic to the integrity of cellular membranes and to membrane bound enzymes (Bus and Gibson 1979). Kartha and Krishnamurthy (1978) reported that among the different tissues, the brain showed considerably high degree of peroxidation. Reaction of oxygen with lipids leads to a series of molecular rearrangements termed as peroxidation which results in a series of oxidative derivatives, including lipid peroxides, lipid hydroperoxides and aldehydes (Enterbaver 1982). Some of these are toxic to various cells, including endothelial cells (Sasaguri et al 1984, Peng et al 1985). Disruption of lysosomal membranes by lipid peroxidation can spill hydrolytic enzymes into the rest of the cell and thus potentiate the damage.

Systemic study of the reaction kinetics of lipid peroxidation has shown that the process is rather complicated (Vladimirov & Archakov 1972). The chemical

process of lipid peroxidation (Mead 1976, Pryor et al 1976) is defined as the reaction of an oxidant initiator with a polyunsaturated fat (LH) to form a lipid-free radical intermediate (L*) leading to a peroxy free radical (LOO**) when it reacts with oxygen. A relatively non-specific hydrogen abstraction reaction is initiated when the unpaired electrons of the peroxy free radical react with another lipid molecule.

$$L^{*} + O \longrightarrow LOO^{*}$$
$$LOO^{*} + LH \longrightarrow LOOH + L^{*}$$

The peroxy free radical can also react with an antioxidant to terminate the reaction chain. The administration of exogenous antioxidants not only inhibits lipid peroxidation but also restores the high level of thiols because of the lower concentration of hydroperoxides formed in the chain reaction.

A mechanism for lipid peroxidation was proposed by Dahle et al (1962) which attempts to explain the following observations: the increase in absorbance at 233 nm at the early stage of oxidation, the appearance of lipid peroxides as intermediates in the reaction, the inhibiting effect of chain-breaking antioxidants on the reaction, and the more facile production of malonaldialdehyde as quantified by the TBA test from linolenic or arachidonic acids when compared with linoleic acid. The steps in the oxidation of a diene or triene fatty acid systems are depicted in Fig.1.11. This free

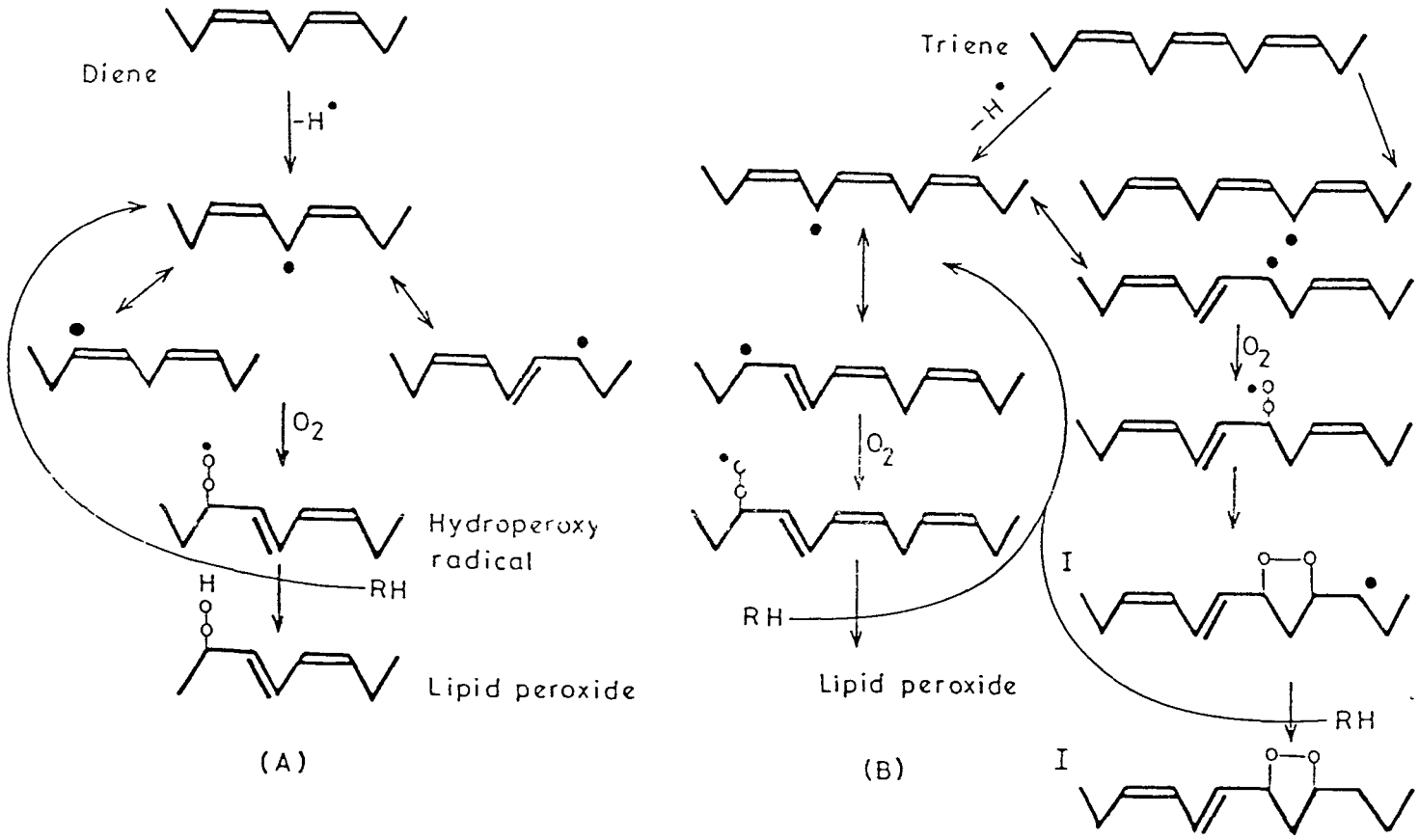
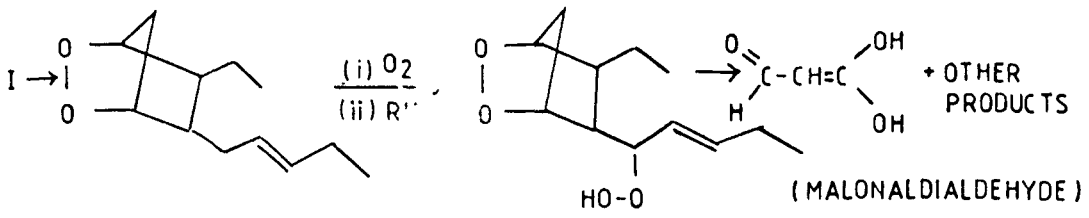


Fig.1-11: Oxidation of a diene (A) and a triene (B) fatty acid system adapted from the mechanism proposed by Dahle et al. (1962). I the triene mechanism the oxidation of only one of the fatty acid radicals initially produced by hydrogen abstraction is shown. The oxidation of the other free radical is similar to the mechanism presented. RH, a polyunsaturated fatty acid.

radical chain reaction is initiated when some unidentified free radical abstracts a methylene H atom from the unsaturated fatty acid. The resulting free radical is stabilized by resonance, with several of the resonance forms adopting a conjugated diene system. Oxygen adds to the lipid free radical producing a hydroperoxy radical which may abstract a hydrogen atom from another unsaturated fatty acid, thereby propagating the chain reaction, to form a lipid peroxide. According to Dahle et al (1962) malonaldialdehyde is produced by the triene system, and not the diene system, because only with the triene is it possible to obtain the hydroperoxy radical with a double bond between the carbons B, r to the peroxy radical. It is possible to obtain the cyclic peroxide II in triene systems which is the non-volatile precursor of malonaldialdehyde. Since both linoleic and linolenic acids can form II, then Dahle's mechanism does not explain the difference in the rate of malonaldialdehyde formation from linoleic and linolenic acids. Pryor et al (1976) suggest that the fatty acid free radical I abstracts a hydrogen atom internally. Triene systems produce a more stable bicyclic free radical III than diene systems since they are stabilized by the third double bond.



The endoperoxide IV is the non-volatile malondialdehyde precursor in Pryor's mechanism. Pryor et al (1976) have also shown that many cyclic peroxides produced during the oxidation of unsaturated fatty acids give positive TBA tests.

From this laboratory, various studies have demonstrated alterations in the rate of lipid peroxidation in the CNS regions following exposure to organophosphate pesticides (Islam et al 1983; Tayyaba & Hasan 1985; Hasan & Khan 1985; Vadhwa and Hasan 1986, Naqvi et al 1988), toxic gases and heavy metals (Haider & Hasan 1984, Bano and Hasan 1989, Farahani and Hasan 1990) and ageing (Gupta & Hasan 1988). No report, however, is available on the effect of dimecron on the brain lipid peroxidation.

1.9.0 Glutathione-S-transferase

Glutathione-S-transferases (GSTs) are a group of widely distributed (Grover and Sims 1964, Bend et al 1978, Mukhtar et al 1981) and multifunctional enzymes, which play a crucial role in the response of cells to a vide variety of acute and chronic toxins (Patridge et al 1983, Stockstill & Dauterman 1982). Following types of GSTs have been describe so far:

(1) Glutathione-S-alkyl transferase, catalyzing the conjugation of a variety of alkyl halides with glutathione (Johnson 1966).

(2) Glutathione-S-epoxide transferase, active towards the conjugation of active epoxides with glutathione (Boyland & Williums 1965).

(3) Glutathione-S-alkene transferase, catalyzing the conjugation of unsaturated compounds with glutathione.

Glutathione (GSH) is a nucleophile involved in conjugation reactions which are important for elimination of foreign compounds in the body, such as electrophilic toxicants (Chasseaud 1980). The conjugates which are formed with GSH are more water soluble. GSTs are thought to play a physiological role in initiating the detoxification of potential alkylating reagents including toxicants and other pharmacologically active compounds (Boyland & Chasseaud 1969, Wood 1970). These enzymes catalize the reaction of such compounds with sulfhydryl (-SH) group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water soluble (Habig et al 1974). Glutathione-S-conjugates formed by GST are further catabolized (Fig.1.12) to cysteine derivatives by simultaneous removal of glutamyl and glycine group by enzymes, r-glutamyl transferase and cystinyl glycinase respectively. S-conjugates are further acetylated by N-acetyl transferase to mercapturic acid (Boyland & Chasseaud 1969).

Jakoby and Keen (1977) have proposed that GST provides a 'triple treat' in the detoxification reactions: firstly, as

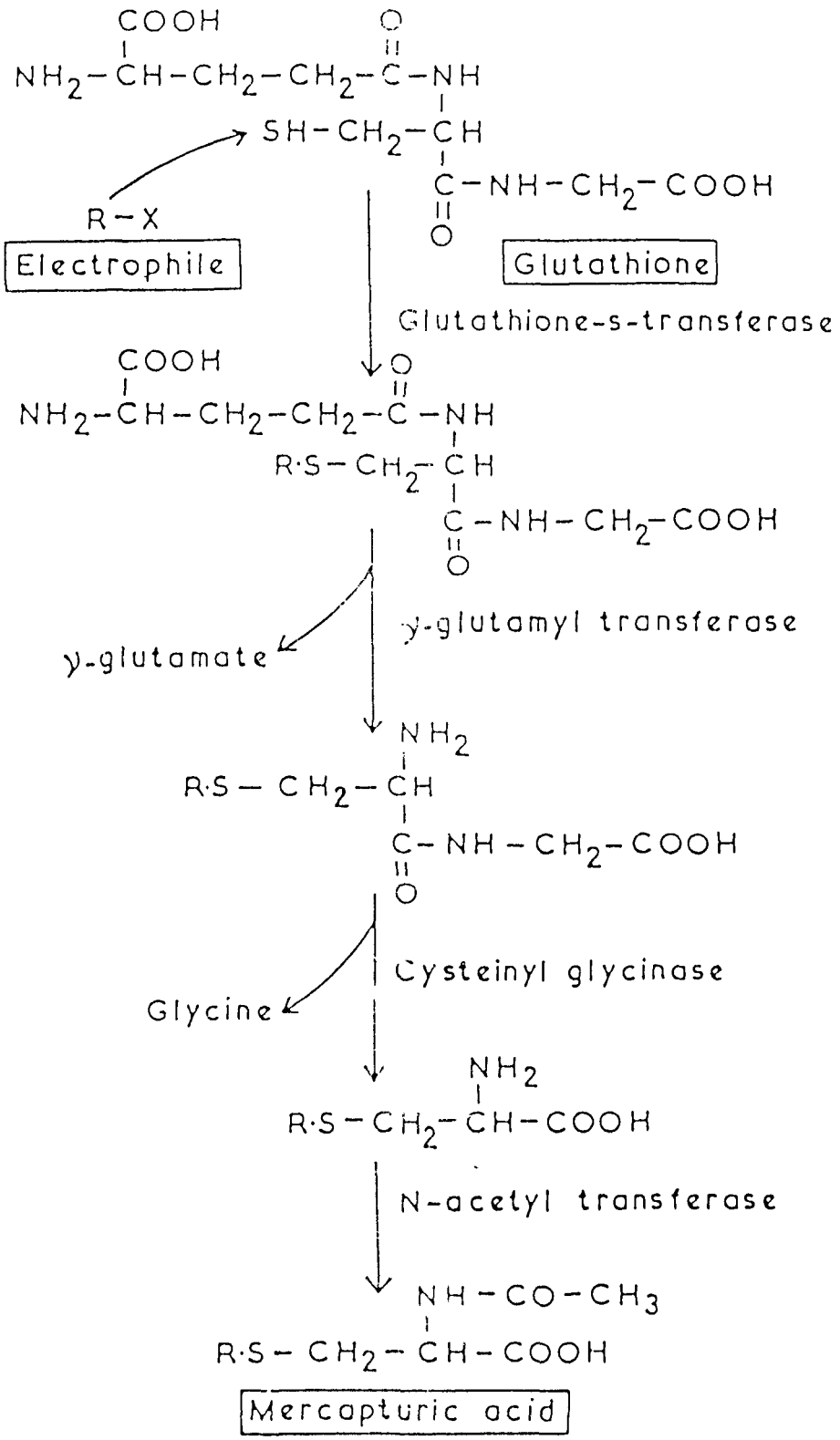


Fig.1·12 : Role of glutathione-s-transferase in synthesis of mercapturic acid

a catalysts in many reactions in which GSH acts as nucleophile, secondly as a storage facility for various compounds prior to their metabolism or excretion and thirdly as "sacrificial" covalent linkage for elcectrophilic compounds.

Numerous compounds which can be detoxicated through GST include pesticides, food preservatives, food additives, carcinogens, polycyclic aromatic hydrocarbons, aminoazodyes (Boyland & Chasseaud 1969, Smith et al 1977). The currently available literature lacks any report regarding neurochemical effects of dimecron on the GST activity.

1.10.0 Sulfhydryl Groups

Sulfhydryl (-SH) groups play a pivotal role in many important enzymes by acting as active enzymatic sites (Hoch and Vallee 1959). Sulfhydryl groups derived from the side chains of cysteine residues, occur in a number of enzymes. There are two major types of -SH groups viz. protein-bound (PB) and non-protein bound (NPB).

Sulfhydryl groups play an important role in GST-induced detoxification against electrophilic xenobiotics and toxicants by conjugating with such compounds and thus neutralizing their electrophilic sites (Habig et al 1974). NPB-SH has got a vital role in the cellular detoxification against free radical-mediated damage (Chio & Tappel 1969). Sulfhydryl enzymes have been found to be most susceptible to

lipid peroxidation-induced inactivation. Tappel (1970) has suggested that deficiency of total and free (NPB) -SH groups may lead to deficient degradation of lipid peroxides to hydroxy radicals, resulting in the accumulation of peroxides in various regions of the brain. The association of glutathione with lipid peroxidation is well recognized (Tappel and Dillard 1981).

Sulfhydryl group of cysteine and the disulfide bond (-SS-) of cysteine are highly reactive and apparently involved in the maintenance of the conformation and biological activity of certain proteins. As the receptors are protein in nature, the reagents which modify -SH groups may influence the interactions of neurotransmitters with their recognition sites (Sobrino & Del Castillo 1972, Barrantes 1980).

Owing to the important role of -SH groups against the toxic compounds, it is relevant to evaluate the effect of organophosphate dimecron on the brain -SH contents.

1.11.0 Superoxide dismutase

Superoxide dismutase (SOD) is a very important enzyme which is essential for the survival of aerobic cells. Superoxide radical (O_2^-) is a common intermediate of oxygen reduction. O_2 is readily generated by so many spontaneous and enzymatic oxidations that they may be assumed to be produced within all respiring organisms (Misra & Fridovich 1972;

Heikkila & Cohen 1973, Maklund & Marklund 1974). Catalytic actions of several enzymes have been shown to evolve O_2 (McCord & Fridovich 1969, Massey et al 1969, Rajagopalan & Fridovich 1962, Rajagopalan & Handler 1964). Superoxide dismutase is the enzyme which catalytically scavenges the superoxide radical, which appears to be an important agent of the oxygen toxicity, and hence provides a natural defence mechanism against oxidative damage to the tissues (Fridovich 1975). The reaction whose rate it enhances is a disproportionation or dismutation of O_2 radicals and may be expressed as:

$$O_2 + O2 + 2\ H^+ \longrightarrow H_2\ O_2 + O_2$$

SOD is inducible by oxygen (Gregory & Fridovich 1973a, Gregory & Fridovich 1973b, Gregory et al 1974). This induction allows manipulation of the intracellular level of the enzymes by variation of the oxygen tension under which the cells are grown. Studies on both micro-organisms, as well as higher organisms, establish that SOD serves as an essential defense mechanism against oxygen and superoxide toxicity (Michelson & Buckingham 1974, Crapo & Tierney 1974). SOD has been reported to prevent the swelling of rat liver mitochondria induced by glutathione.

Organophosphates have been found to cause oxidative damage to the tissues through lipid peroxidation (Tappel & Dillard 1981, Islam et al 1983; Tayyaba & Hasan 1985; Demopoulos et al 1979; Hasan & Khan 1985, Vadhva & Hasan

1986, Naqvi et al 1988). It is, therefore, possible that SOD might also play an important role in the organophosphate toxicity. No report, however, is available todate, on the effect of organophosphate pesticides on the SOD activity.

1.12.0 Nucleic acids

The discovery of the nucleic acids was the result of the work of Friedrich Miescher (1844-1895), the founder of our knowledge of the chemistry of the cell nucleus. In 1871 he published a paper regarding the isolation of an acidic compound "nuclein" from the nuclei of the pus cells, which was readily soluble in dilute alkali but insoluble in dilute acid, and contained a high proportion of phosphorus (Miescher 1871). A number of scientists followed him, but only in 1930, a definite picture of two definite types of nucleic acids had emerged. One of them, the nucleic acid isolated from yeast, on hydrolysis yielded adenine, guanine, cystosine, uracil, phosphoric acid and a sugar (pentose), was identified by Levene and Jacobs (1909) as ribose. The other, the nucleic acid from thymus, yielded adenine, guanine, cytosine, thymine, phosphoric acid and a sugar (deoxypentose), was identified as deoxyribose (Levene et al 1930). The two nucleic acids, therefore, came to be called ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), respectively. Initially, an assumption was made that DNAs were characteristics of animal tissues and RNAs, of plant tissues, but later on RNA was found to be present in animal tissue as

well (Jorpes 1934).

In the brain, the nucleic acids provide for the storage and transmission of genetic information as well as information leading to the synthesis of cellular proteins (White et al 1978).

A knowledge of DNA content helps in understanding the tissue components such as average cell densities, dry weight/ average cell and total number of cells in each brain area (May & Grenell 1959). The only well-authenticated sites at which DNA is found in all animal cells, including the brain, are the nucleus (including nucleolus) and mitochondria (Mahler 1981). The DNA content of diploid mammalian cells, including neurons and other brain cells, is of the order of 6.5 picograms per cell (or about 1 microgram per milligram of wet brain weight). This corresponds to a mass of 3.8×10^{12} daltons for the total chromosomal complement of such cells. Thus the maximal (haploid) content of genetic information available to these cells is about 800 times that of E. Coli, or 100 times that of yeast, encoded in 1.5×10^{9} base pairs. The amount of DNA in white matter approximately equals that in the cortex, and the regional differences in the content of brain DNA are relatively small (Logan et al 1952; Elliott & Heller 1957). Cerebellum, however, shows exceptionally high amounts of DNA (Grenell 1958, May & Grenell 1959). Deoxyribonuclease (DNase) is a highly specific

phosphodiesterase enzyme responsible for the hydrolytic degradation of DNA to mononucleotides and subsequently to nucleotides (Schmidt 1955). Neuronal nuclei prepared from young and adult rat brains have been shown to have a higher DNase activity than glial nuclei (Stanmbolova 1973).

It has been reported that organophosphate, DDVP, induces degenerative changes in neurons and nerve fibres (Hasan et al 1979). The loss of myelin during degeneration and the subsequent myelination of the newly growing fibres bring about changes in most of the cell constituents including nucleic acids (Mcllwain & Bachelard 1971). Organophosphate metasystox has been reported to deplete the concentration of DNA in various brain regions, duly correlated with increased activity of DNase (Tayyaba et al 1981).

In every cell not infected by an RNA virus, all RNA synthesis is absolutely dependent on the presence of a DNA template and is catalysed by a family of enzymes called DNA-dependent RNA polymerases (Chambon 1974). Therefore RNA synthesis is expected to be localized at only two intracellular sites in all cells of the nervous system: in the nucleus, including the nucleolus, and in the mitochondria (Chambon 1978). Most of the RNA of neurons is localized in the perikaryon, and there, as in all cells, the majority forms an integral constituent of ribonucleoprotein particles that are themselves aggregated into polymeric structures.

These structures exist either free in cytosol or attached to membranes of endoplasmic reticulum. They are held together by messenger RNA (m RNA), and in conjunction with the soluble transfer RNA (tRNA) of cytosol, constitute the protein-synthesizing machinery of the cell. In addition to their peikarya, certain neurons also may contain some RNA in their axons, including the presynaptic thickening. The axonal RNA may contribute some 0.5 mg per gram of wet weight (Koenig 1969). The studies of RNA content helps to assess the rate of protein synthesis and also to evaluate the functional status of the nervous system (Bergen et al 1974). Edstrom (1956) and Edstrom and Pigon (1958) have suggested a proportionality between RNA content and the surface area of the cell body. It has been suggested that, in addition to the established role of nucleic acids in the biosynthesis of protein (Campbell 1965), RNA and protein synthesis may be involved in the accrual of sensory information in the brain, thus indicating a possible approach to elucidation of brain function on molecular basis (Hyden 1964).

Amount of RNA in gray matter usually exceeds that in white matter (Mihailovic et al 1958, Grenell 1958). RNA is highly concentrated in the nucleolus and in the Nissl substance of the cytoplasm of nerve cells (Landstrom et al 1941; Hyden 1943). Ribonuclease (RNase), a highly specific phosphodiesterase enzyme, hydrolyzes all known RNAs by

catalyzing the cleavage of certain strictly defined internucleotide bonds (Schmidt 1955).

It has been reported that RNA content was found to increase in the rat brain following metasystox toxicosis (Tayyaba et al 1981). It was demonstrated that RNA synthesis increases due to damage of the neurons, in which, RNA level remains high between 10 to 30 days after which it returns to normal level (McIlwain & Bachelard 1971).

The available literature, however, lacks in any report on the neurochemical alterations in the metabolism of nucleic acids following dimecron toxicosis.

1.13.0 Proteins

Proteins are one of the many important biochemical components of the vertebrate brain. They constitute 40% of the dry weight and 8% of the wet weight of the fresh brain (McIlwain & Bachelard 1971). Proteins specific to the nervous system are of interest because they underline the developmental specialization and differentiation of the CNS cells. Some of the important nervous system specific proteins are S-100 protein, glial fibrillar protein, myelin proteins, postsynaptic density proteins, receptors for neurotransmitters, enzymes involved in catecholamine synthesis, etc. (Mahler 1981). The susceptibility of brain proteins, especially those localized in the synaptic region and its membranes, to covalent modification may provide a

means for altering the efficacy of synaptic transmission as a function of neuronal activity. As the brain has high rate of metabolic activities, it needs more proteins for expected high rate of protein turnover. This view is well in agreement with the presence of large amount of cytoplasmic ribosomes which provide large number of sites for protein synthesis (McIlwain & Bachelard 1971).

The changes in the neuronal activity are found to be accompanied by perturbations in macromolecules like protein in the brain cells. Increased neuronal activity has been reported to inhibit the protein synthesis (Hyden and Lange 1972). The specific neuronal functions such as conduction of action potentials and synaptic transmission are extensively mediated by proteins (Bock 1978). Proteins in the brain are in a dynamic steady state. Protein synthesis probably starts from free amino acids, which are activated and transferred to a soluble ribonucleic acid or nucleoprotein (Mahler 1981). Proteins destined for intracellular use are synthesized by polysomal arrays in the cytosol, that are unattached to membranes. Proteins destined for export from the cell of origin as well as those plasma membrane proteins that will become part of its external aspect, are synthesized by polysomes attached to the membranes of endoplasmic reticulum.

Protein contents have been found to diminish in various CNS region following intoxication by phosalone (Tamilvanan

1984), metasystox (Tayyaba et al 1981), methyl parathion (Khan 1989) and dichlorvos (Vadhva 1989). To date, however, dimecron has not been reported to induce changes in the protein metabolism.

2.14.0 AIMS OF THE PRESENT STUDY

Main aims of the present investigation were as follows:

(1) To study quantitative alterations in the following neurochemical parameters in various CNS regions of dimecron-intoxicated rats:

- Acetylcholinesterase activity
- Concentration of monoamines
- Monoamine oxidase activity
- Concentration of total lipids, phospholipids, cholesterol and gangliosides
- Rate of lipid peroxidation
- Content of sulfhydryl groups
- Activity of glutathione-S-transferase
- Activity of superoxide dismutase
- Level of nucleic acids and total protein

(2) To evaluate dimecron - induced perturbation in the Open Field Behaviour of the treated rats, and to correlate them with the alterations in the monoamine metabolism.

(3) To determine the quantity of the dimecron accumulated in different regions of the CNS following graded dosing of dimecron, and to find out possible correlation between accumulated dimecron content with its neurotoxicity.

(4) To investigate the possible protective effect of antioxidant, acetylhomocystein thiolactone against the neurotoxic effects of dimecron.

2. MATERIAL AND METHODS

2.1 Animals:

Male albino rats of Charles Foster strain, weighing 150 ± 20 g, were obtained from the Central Animal House of J.N. Medical College, A.M.U., Aligarh. They were fed commercial diet (Hindustan Lever Laboratory, Bombay) and water ad libitum and maintained in normal laboratory conditions with a light-dark cycle of 12:12 hrs.

2.2 Drugs used:

1. Dimecron: Dimecron (2-chloro-2-diethylcarbamoyl-1-methylvinyl dimethyl phosphate), commonly known as phosphamidon, was procured from Hindustan CIBA-GEIGY Ltd, Bombay. Intraperitoneal LD_{50} of dimecron for rats has been reported to be 5.8 mg/kg (Menzer and Best 1968).

2. Cithiolone: Cithiolone (DL-N-acetylhomocysteine thiolactone) was purchased from Aldrich Chemical Company Inc, USA.

2.3 Experimental Design:

Animals were broadly divided into four groups. To animals of the first group, serving as experimentals, freshly prepared dose of dimecron (2.0 mg/kg b. wt) was administered intraperitoneally (ip) daily for seven days. The dose was prepared by dissolving 0.023 ml of commerical dimecron (85% v/v) in 10 ml physiological saline (0.9% sodium chloride). The second group served as 'control' and was given equal volume of saline (ip) concurrently. The third and fourth

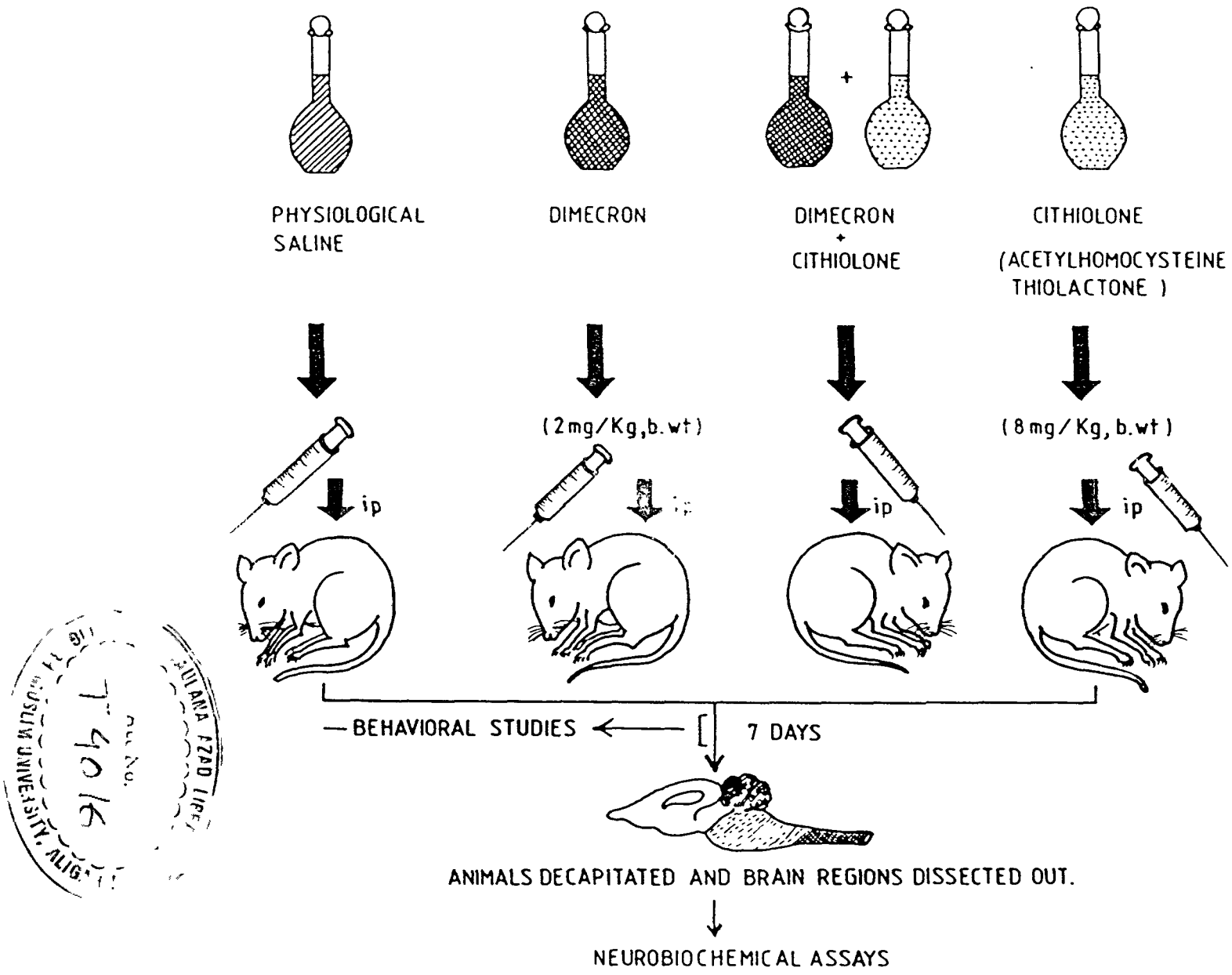
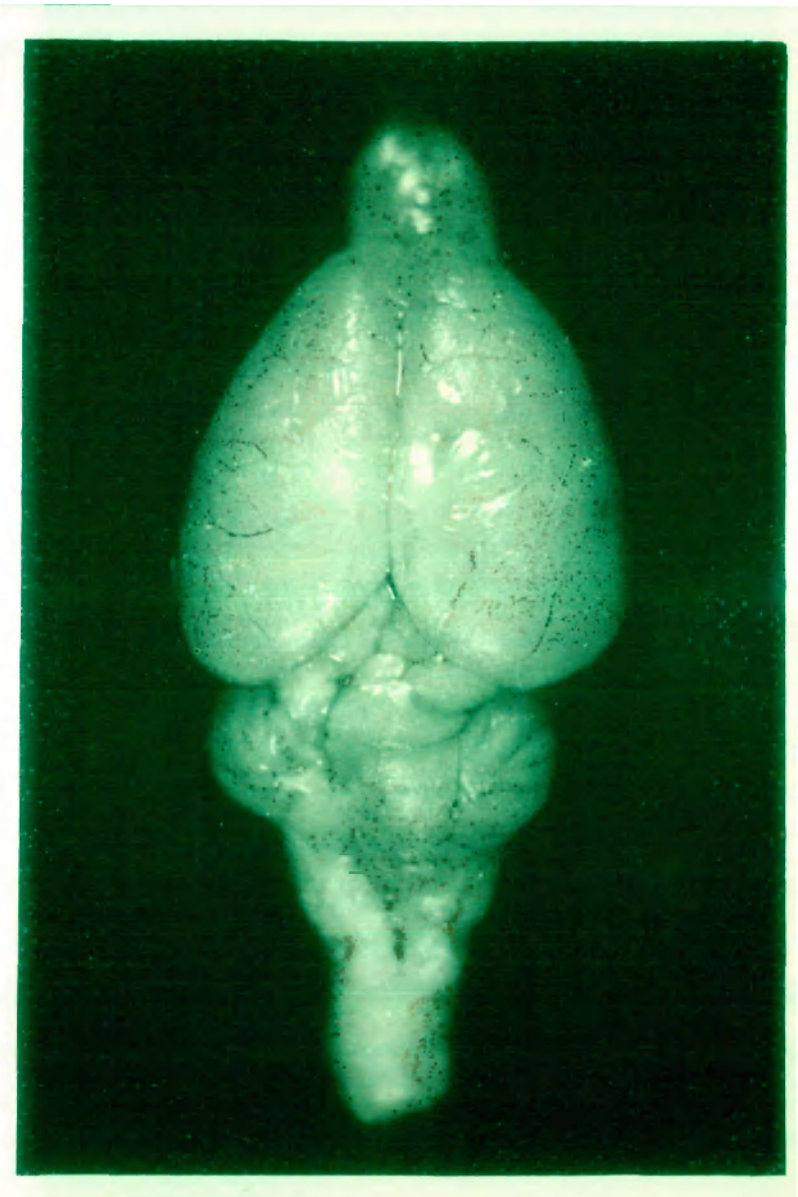


FIG. 2·1. SUMMARIZED SCHEMATIC REPRESENTATION OF EXPERIMENTAL DESIGN.

Fig. 2.2 Photograph of dissected rat brain (Dorsal view)



groups were used in the studies related to the protective effects of cithiolone against dimecron neurotoxicity. Animals of the third group received cithiolone (8.0 mg/Kg, b.wt, ip. alone), while the fourth group was administered dimecron (2.0 mg/Kg b. wt, ip) and cithiolone (8.0 mg/Kg b. wt, ip) concomitantly for seven days. The dose of cithiolone, chosen after Totaro et al (1968), was prepared by dissolving 80 mg commercial cithiolone (99%) in 10 ml physiological saline.

[A] BEHAVIORAL STUDIES

2.4 Open Field Behaviour

2.4.1 Apparatus: The apparatus used in the open field behaviour (OFB) test was similar to that used by Holland and Gupta (1966). Briefly, it consisted of a wooden, circular open arena (82 cm diameter) surrounded by a wall 31 cm high. The wooden floor was marked with three centric circles which were divided into segments by lined radiating from the centre. These 25 units of approximately equal size were used to score ambulation of the animals during the test. Two types of stimuli were presented to the animals: white noise (78 dB, Ref. Intensity 0.0002 dyn/cm^2) was produced by an oscilator through four loudspeakers; and light (165 footcandles) was presented by four lamps. A translucent glass screen enclosed the arena on all sides, the front side having a glass door through which the animals were placed in the arena.

2.4.2 Procedure: After one hour of injections, each animal of both the experimental and control groups was exposed daily

for two minutes in the open field chamber, and the ambulation, rearing and preening responses were recorded by a three-channelled hand-operated counter. The ambulation score was defined as the walking score derived from the number of radial segments crossed by the animals. The placement of all the four limbs in one segment was taken as one unit of ambulation. A rearing score of 1 was awarded when the rat stood on its hind limbs with the support of the wall, and 2 for standing without support. Preening response was determined by the number of times the animal scratched its face with the forelimbs.

[B] BIOCHEMICAL STUDIES

2.5 Dissection and Isolation of Different CNS Parts:

Overnight fasted rats were scarificed on the seventh day of injection, by cervical disolocation followed by decapitation. Brains were removed quickly and the cerebrum, cerebellum, brain stem and spinal cord were dissected out rapidly on ice-plate. Different CNS regions were then cleaned with normal saline to remove the blood clottings, and weighed accurately to the nearest milligram.

2.6 Assay of Acetylcholinesterase

The activity of acetylcholinesterase (AChE) was measured by the method of Ellman et al (1961).

The principle of the method is the measurement of the rate of production of thiocholine as AChE is hydrolysed. This

is accompanied by the continuous reaction of the thiol with 5,5-dithiobis-2-nitrobenzoate ion to produce the yellow anion of 5-thio-2-nitrobenzoic acid.

2.6.1 Chemicals and Reagents:

1. Phosphate buffer (0.1 M, pH 8.0)
2. DTNB Reagent: 39.6 mg of 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was dissolved in 10 ml of 0.1 M phosphate buffer (pH 8.0), and 15 mg of soidum bicarbonate was added to it.
3. Substrate: 0.075 M acetylthiocholine iodine (Sigma, USA).
4. Inhibitor: 10^{-4} M eserine sulphate (Sigma, USA) was dissolved in 0.1 M phosphate buffer (pH 8.0).

2.6.2 Procedure: Saline cleaned and accurately weighed tissue were homogenized in 0.1 M phosphate buffer (pH 8.0) in a concentration of 10 mg/ml and centrifuged at 1,500 rpm for 5 min. 0.4 ml of supernatant was pipetted in a cuvette containing 2.6 ml of phosphate buffer. To this, 0.1 ml of DTNB reagent was added and mixed well. The cuvette was placed in a Beckman DU-6 spectrophotometer and the absorbance at 412 nm of the suspension was set to zero. 0.02 ml of the substrate was added and the changes in the absorbance were recorded from 5th to 10th min at the interval of one minute. To determine non-specific esterase, 0.1 ml of eserine sulphate was added to another cuvette containing 0.4 ml of homogenate supernatant, 2.5 ml of phosphate buffer and 0.1 ml DTNB reagent. The changes in absorbance were recorded as described earlier, after adding 0.02 ml of substrate. The rate of change of activity of the suspension with eserine was

substracted from that of the suspension without eserine. The enzyme activity is expressed as μ moles of substrate hydrolysed per g tissue per minute.

Calculation:

$$R = \frac{A}{1.36(10^4)} \times \frac{1}{(400/3120)Co} = 5.74\ (10)^{-4}\ \frac{A}{Co}$$

where, R= Rate of enzyme acitivity in moles of substrate hydrolysed/g tissue/ min

A = Change in absorbance per minute

Co = Original concentration of tissue (mg/ml)

2.7 Determination of Monoamines

Monoamines, dopamine (DA), norepinephrine (NE) and serotonin (5-HT) were extracted and estimated according to the method of Welch and Welch (1969).

2.7.1 Chemicals and Reagents:

1. n-Heptane: Analytical Grade
2. n-Butanol: Analytical Grade
3. Peroxide free ether: Diethyl ether was washed with saturated solution of ferrous sulphate to remove accumulated peroxides. Thereafter, washed with double distilled water (DDW) to remove the excess of sulphate.
4. Ethylenediamine
5. Acetate Buffer (2 M, pH 6.8): 2M acetic acid was adjusted to pH 6.8 with 2N sodium hydroxide.
6. Phosphate Buffer (0.5 M, pH 7.3): 77 ml of 0.5 M monobasic sodium phosphate was mixed with 23 ml of 0.5 M dibasic sodium phosphate.

7. Sodium thiosulphate (0.1N): 1.581 g of sodium thiosulphate ($Na_2 S_2O_3.5H_2O$) was dissolved in 100 ml DDW.
8. EDTA solution (10%): 10 g of ethylenediaminetetraacetic aid (EDTA) was dissolved in 100 ml DDW.
9. Alkaline sulphite-EDTA solution: 12.6 g sodium sulphite was dissolved in 100 ml 10% EDTA solution and diluted to 250 ml with 5N NaOH.
10. Alkaline Ascorbic acid-Ethylenediamine solution: 200 mg of ascorbic acid was dissolved in 25 ml of 0.01 N HCl and added to a mixture of 0.5 ml ethylenediamine and 22.5 ml of 10 N NaOH. This was thoroughly mixed by shaking. This reagent was freshly prepared immediately before use.
11. Iodine solution: 3.175 g iodine together with 12.5 g of sodium iodine were dissolved in 250 ml of DDW, and stored in a dark-coloured bottle in cold.
12. Stock solutions: The solutions of dopamine, norepinephrine and serotonin were prepared in 0.01 N HCl at a concentration of 100 µg/ml and stored in refrigerator.
13. Standard solutions: On the day of experiment, the stock solutions were diluted with 0.01 N HCl to get working standard solutions corresponding to 100 ng/ml of NE and 200 ng/ml of DA and 5-HT.

2.7.2 Extraction Procedure: The brain parts were homogenized in 1.5 ml ice-cold 0.01 N HCl. 0.1 ml of 10% EDTA was added to the homogenate and then transferred to glass-stoppered bottles containing 2.5 ml of n-butanol and 2 g of NaCl. The bottles were shaken for 10 min and centrifuged at 3,000 rpm for 10 min. in cold. The butanol layer was separated, transferred to another set of glass-stoppered botles each containing 20 ml

n-heptane and then added 1.5 ml phosphate buffer. The mixture was shaken for 10 min and centrifuged in cold, keeping pH not below 7. The phosphate buffer (1.5 ml) was drawn from butanol phase and transferred to another bottle and pH was adjusted to 3.5-4 using 3N HCl. Thereafter, 10 ml of peroxide free ether was added and bottles were shaken well and centrifuged in cold. The acidic aquous layer was taken from bottom with 0.5 ml micropipette and three 0.5 ml aliquots were collected separately for the determination of DA, NE and 5-HT respectively.

2.7.3 Analysis of Monoamines: The samples of monoamines obtained after extraction, were analysed using RF-40 spectrofluorometer Shimadzu, Japan according to the following procedures:

2.7.3.1 5-HT: The sample (0.5 ml) was mixed with 0.5 ml 6N HCl and the native fluorescence was immediately read at 295/535 nm using 5 mm slit. Thereafter these tubes were used as blanks for DA analysis.

2.7.3.2 Norepinephrine: To each 0.5 ml extract, 0.5 ml 2 M acetate buffer, 0.1 ml iodine solution, 0.15 ml 0.1 N sodium thiosulphate and 0.2 ml alkaline ascorbic acid-ethylenediamine mixture were added in respective order, thereby making a total volume of 1.45 ml. Each reagent was added after an interval of 5 min and the viscous mixture was thoroughly mixed by shaking. The fluorescence was recorded at

400/510 nm using 2 or 3 mm slit.

2.7.3.3 Dopamine: 0.5 ml extract was mixed at 5 min intervals with 0.5 ml 2 M acetate buffer, 0.1 ml iodine solution, 0.2 ml alkaline sodium sulphite-EDTA solution and 0.25 ml 1:1 glacial acetic acid-HCl mixture, respectively to make a final volume o 1.5 ml. The tubes were placed in boiling water bath for 45 min and then allowed to cool at room temperature. Fluorescence was read within 1-2 hr at 335/380 nm using 2 mm slit.

2.7.4 Preparation of standard curves: The standard curves for DA, NE and 5-HT were prepared by analysing fluorometrically (as described in "Analysis of Monoamines"), the standard amine solutions in the range of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, ml corresponding to 10, 20. 30, 40, 50, 60, 70 ng of NE and 20, 40, 60, 80, 100, 120, 140 ng of each of DA and 5-HT.

2.8 Assay of Monoamine Oxidase

The activity of monoamine oxidase (MAO) was assayed following spectrophotometric method of Tabor et al (1953).

The benzylamine undergoes oxidative deamination in the presence of MAO leading to formation of benzaldehyde.

2.8.1 Chemicals and Reagents:

1. 0.5 M Phosphate Buffer (pH 7.2)
2. Perchloric acid, 10% (Analytical Grade)
3. Substrate: 0.1 M benzylamine hydrochloride

2.8.2 Procedure:

The reaction mixture in a final volume of 2 ml consisted of 0.4 ml 0.5 M phosphate buffer (pH 7.2), 0.1 ml of 0.1 M benzylamine hydrochloride and 0.2 ml of brain homogenate (10% w/v in phosphate buffer). The reaction mixture was incubated at 37°C for 30 min. The protein was precipitated by centrifuging at 2,500 rpm for 10 min. The optical density (OD) of benzaldehyde formed was read in the supernatant at 250 nm on Beckman DU-6 UV/VIS spectrophotometer against blank treated similar to samples, containing 0.2 ml of 0.44 M socrose instead of the brain homogenate.

Calculation: Activity of MAO = $\frac{15.385 \times OD}{\text{Protein conc.}}$

Where OD = Change in OD per minute

The enzyme activity is expressed as n moles of benzaldehyde formed/ min/ mg protein. Protein was estimated by the method of Lowry et al (1951).

2.9 Brain Lipids

2.9.1 Extraction of Lipids from Discrete Brain Areas:

Different parts of the brain (50-200 mg) were homogenized in a glass homogenizer to a final volume of 6 ml chloroform - methanol (2:1, v/v) according to the method of Folch et al. (1951). Each homogenate was shaken periodically for an hour and filtered through sintered glass funnel (G-4)

under vacuum. The residue of each test tube was again homogenized with 2 ml chloroform-methanol and filtered. The test tubes were rinsed with fresh chloroform-methanol and again filtered. The final volume of each extract was made upto 10 ml with fresh chloroform-methanol mixture. Thereafter 2.5 ml of normal saline solution was added to the extract in each test tube (4:1, v/v). This was shaken vigorously on cyclo-mixer for complete mixing, and placed at -20°C in a deep freeze overnight for complete separation of the two layers. The junction of the two layer in each test tube was marked. The upper layer was used for the estimation of gangliosides. The lower layer was collected in stoppered tubes with the help of a syringe and stored at -20°C until final use. The test tubes in which the two layers were separated, were dried and the volume of the lower layer in each test tube was measured. This extract was used for the estimation of total lipids, phospholipids and cholesterol.

2.9.2 Estimation of Total Lipids:

Total lipids were estimated according to the method of Woodman and Price (1972), described as follows:

2.9.2.1 Chemicals and Reagents

1. Standard Solution: Standard solution of 0.5 mg brain lipids/ml of chloroform-methanol (2:1) was prepared by diluting 1.0 ml refrigerated stock solution (50 mg brain lipid/10 ml chloroform-methanol) in a 10 ml standard flask and made up the volume to 10.0 ml with chloroform-methanol (2:1, v/v).

2. Coloring Reagent: 6.0 g potassium dihydrogen orthophosphate and 1.3 g vanillin were dissolved by heating in a 100 ml volumetric flask and the volume was made upto 100.0 ml with double distilled water (DDW).
3. Conc. Sulfuric acid, A.R.
4. Brain Lipids: Lipids were isolated from the rat brain by the technique as described in the Extraction in Brain Lipids. The extract was dried by vacuum rotatory evaporator at 40°C and dried lipids were stored at 0-5°C.

2.9.2.2 Procedure: 0.1 ml of duplicate brain extracts and a duplicate set of standard with 50 to 500 μg of brain lipids were taken in 18 x 150 mm Corning test tubes. To this was added 2.5 ml conc. sulfuric acid and heated in boiling water bath for 20 min. After cooling, 5.0 ml coloring reagent was added and absorption was read at 530 nm exactly after 10 min against a reagent blank on a Beckman DU-6 spectrophotometer.

Calculation:

$$\text{Total lipids (mg/g fresh weight)} = \frac{C \times V}{Vt \times Wt}$$

where C = Concentration of lipids in g in 0.1 ml brain extract

V = Total volume of lower layer

Vt = Volume taken for estimation

Wt = Fresh weight of the tissue in mg.

The above formula was used also for calculating the concentrations of phospholipids and cholesterol.

2.9.3 Estimation of Phospholipids:

Phosphate of the phospholipids was estimated by the method of Fiske and Subbarow, as described by Marinetti et al. (1962).

2.9.3.1 Chemicals and Reagents:

1. A standard of 0.01 mg inorganic phosphate/ml was prepared by diluting 5.0 ml refrigerated stock solution (0.439 g KH_2 PO_4/500 ml DDW) in a 100 ml standard flask with DDW.
2. Perchloric acid 70% A.R.
3. Ammonium molybdate solution 2.5%.
4. Reducing Reagent: Reducing reagent was prepared by dissolving 3.0 g sodium bisulphite, 0.6 g sodium sulphite and 0.05 g recrystalysed 1-amino-2-naphthol-4-sulphonic acid (ANSA) in 25 ml DDW. A slight yellow solution thus obtained was stored in amber coloured bottle. The colour is stable for a week at the room temperature.
5. Recrystalization of ANSA: 15.0 g soidum metabisulphite, 1.0 g sodium sulphite (anhydrous) and 1.5 g crude ANSA were dissolved in 100 ml DDW by heating on the boiling water bath. Hot solution was filtered through the filter paper, 1.0 ml conc. HCl was added in the filterate and stirred. Precipitable was filtered wiht suction pump and washed with about 30 ml double distilled water and finally with alcohol till washing is colourless. This purified ANSA was dried in oven at 100°C for 1 hr with least possible exposure to light and was transfered to amber coloured bottle.

2.9.3.2 Procedure: 0.2 ml of duplicate brain lipid extract (Lower layer) was placed in 18 x 150 mm Corning test tubes and all the solvent was dried up by heating on a boiling

water bath. Added 1.0 ml AR grade 70% perchloric acid to the samples and heated on a digester for 30 min or until the sample became clear. After complete digestion samples were cooled to room temperature. Thereafter, 1.5 ml ammonium molybdate, 0.2 ml reducing reagent and 7.0 ml DDW were added with vigorous shaking after each addition. The test tubes were heated on boiling water bath for 7 min. After 30 min. the colour intensity was read at 700 nm. A calibration curve was prepared with 1 µg to 8 µg of phosphorous and 1.0 ml perchloric acid. Blank was prepared with 1.0 ml perchloric acid alone.

Calculation: The absorption is a linear function of the phosphorus content and the amount in unknown sample can be calculated by direct proportion with the absorbance obtained for the standard. The amount of the phospholipid was calculated by multiplying with a factor of 25.

2.9.4 Estimation of Cholesterol

Total cholesterol was estimated according to the method of Henly (1957), slightly modified in accordance with our laboratory conditions.

2.9.4.1 Chemicals and Reagents:

1. Standard Solution: For Stock standard solution, 10 mg cholesterol (BDH) was dissolved in 10 ml chloroform. The working standard was prepared by diluting 1.0 ml of the stock solution to 25 ml chloroform, so that 1 ml of this solution contains 0.04 mg cholesterol.

2. Ferric chloride-acetic acid solution: 0.25 g $FeCl_3.6H_2O$ (ferric chloride) was dissolved in 500 ml glacial acetic acid.
3. Glacial acetic acid: Analytical Grade.
4. Conc. Sulphuric acid: Analytical Grade.

2.9.4.2 Procedure: Brain extract (0.05 ml) was taken in 18 x 150 mm test tubes and placed in boiling water bath until the solvent was completely evaporated. After cooling it down to room temperature, 6.0 ml of ferric chloride solution was added and centrifuged at 3,000 rpm for 10 min. Supernatant was drawn out and 5.0 ml of it was heated on boiling water bath for one minute and cooled at room temperature. Thereafter, 5.0 ml of conc. sulphuric acid was added to each test tube and mixed at once. Exactly after 10 min, the absorbance was read at 560 nm. The standard set was run similarly as described above, and the calibration curve was plotted by least square method.

The concentration of cholesterol was expressed as mg per gramme of fresh tissue.

2.9.5 Estimation of Gangliosides:

Gangliosides were estimated according to the method of Pollet et al. (1978), as follows:

2.9.5.1 Chemicals and Reagents:

1. Standard solution: A standard solution of 100 μg N-acetyl neuraminic acid (Sigma Chem., USA) / ml DDW was made by diluting 1.0 ml of the refrigerated stock

solution (10.0 mg N-acetyl neuraminic acid dissolved in 10 ml DDW) to the final volume of 10.0 ml with DDW.

2. Resorcinol Reagent: It was prepared by mixing 10.0 ml of 3% resorcinol solution in DDW, 80 ml conc. HCl, 0.25 ml 0.1 M copper sulfate and DDW upto 100 ml.

2.9.5.2 Procedure: To 2.0 ml of the upper layer of the lipid extract, 2.0 ml of resorcinol solution was added. The test tubes were heated on boiling water bath for 30 min. After cooling, 5.0 ml of a mixture of n-butyl acetate-n-butanol (85:15, v/v) was added to each tube. The tubes were shaken thoroughly and kept for 15 min to separate the organic phase. About 3-4 ml of the organic phase was taken and absorbance was measured at 580 nm against a reagent blank. A standard curve with different concentrations of N-acetyl neuraminic acid (5-30 µg) having 2.0 ml final volume of DDW was prepared by the similar procedure.

Calcualtion:

$$\text{Ganglioside (mg/g fresh wt)} = \frac{C \times V}{Vt \times Wt}$$

where C = Concentration in g in 2.0 ml extract

V = Total volume of the upper layer

Vt = Volume taken for the estimation

Wt = Fresh weight of the tissue in mg.

2.10 Determination of Rate of Lipid Peroxidation:

The amount of malonaldialdehyde formed / 30 min during lipid peroxidation was estimated according to the procedure of Utley et al. (1967), as described below:

2.10.1 Chemicals and Reagents:

1. 0.15 M Potassium chloride: 2.2368 g KCl dissolved in 200 ml DDW.
2. 10% (w/v) Trichloroacetic acid: 10.0 g TCA dissolved in 100 ml DDW.
3. 0.67% 2-thiobarbituric acid (TBA): This was prepared by dissolving 0.67 g TBA in 25-50 ml DDW by adding two pellets of NaOH. The pH of the solution was adjusted to 7.2 with the help of 1N HCl and the volume was made upto 100 ml with DDW.

2.10.2 Procedure: Different parts of the brain were homogenized (10%, w/v) in chilled 0.15 M KCl. One of each homogenate was taken in a 25 ml conical flask and incubated at 37 $\pm$ 1°C in a metabolic shaker (120 strokes / min; amplitude 1 cm) for 2 hours. Thereafter, 1.0 ml of the same homogenate was pipetted in centrifige tubes and protein was precipitated by adding 1.0 ml of 10% TCA. After incubation, 1.0 ml of 10% TCA was added to each sample and both the incubated as well as non-incubated samples were centrifued at 3000 rpm for 10 min. One ml of the clear supernatent was mixed with 1.0 ml of 0.67% TBA and 1.0 ml DDW and the tubes were placed in a boiling water bath for 10 min, cooled and the absorbance of the colour was measured at 535 nm.

Calculation:

Lipid peroxidation was calculated using the following formula:

$$X = \frac{OD \times 30 \times 1000 \times 1000 \times 1000 \times 3 \times 2}{1.56 \times 100000 \times 1000 \times 180}$$

$$\text{or} \qquad X = \frac{OD \times 10}{1.56}$$

where X = nanomoles of malonaldialdehyde formed / 30 min.

OD = Change of optical density at zero hr and 2 hr incubation of the same sample.

2.11 Sulfhydryl (-SH) Groups:

Sulfhydryl groups were estimated following the method of Sedlak and Lindsay (1968).

2.11.1 Chemicals and Reagents:

1. 0.15 M KCl
2. Methanol: Analytical Grade
3. Tris-EDTA buffer (pH 8.2): 0.2 M tris (2.423 g) and 0.2 M EDTA (0.744 g) were dissolved in 80 ml DDW, pH was adjusted to 8.2 and the volume was made up to 100 ml with DDW.
4. Tris-EDTA buffer (pH 8.9): 0.4 M tris (4.846 g) and 0.02 M EDTA (0.744 g) were dissolved in 80 ml DDW, pH adjusted to 8.9 and the volume was made up to 100 ml with DDW.
5. Dithiobis-2-nitrobenzoic acid (DTNB), 0.01 M: 99 mg DTNB was dissolved in 25 ml methanol.
6. Trichloroacetic acid (TCA): Analytical Grade
7. Standard: Glutathione (reduced)

2.11.2 Procedure:

2.11.2.1 Total -SH: 0.1 ml of the brain homogenate (10% w/v, in chilled 0.15 M KCl) was mixed with 1.5 ml Tris-EDTA (pH 8.2) and 0.1 ml DTNB solution. After shaking well, 8.3 ml methanol was added and the tubes were centrifuged at 6000 rpm

in cold for 5 min. The supernatant was drawn and the yellow colour was read at 412 nm on Beckman DU-6 spectrophotometer within 30 min.

2.11.2.2 Non-Protein bound (free) -SH: One ml of the brain homogenate (10% w/v, in chilled 0.15 M KCl) was mixed with 1 ml 10% TCA and centrifuged at 6000 rpm for 15 min. From the supernatant, 0.5 ml aliquot was taken and 0.5 ml DDW was added to it. This was mixed with 2.0 ml Tris-EDTA buffer (pH 8.9) and 0.1 ml DTNB solution, and shaken well. The absorbance of the yellow colour was recorded at 412 nm within 30 min.

2.11.2.3 Protein bound -SH: Protein bound sulfhydryl groups were determined by substracting free -SH from total -SH, as described by Sedlak and Lindsay (1968).

2.12 Glutathione-S-transferase:

The mitochondrial enzyme, glutathione-S-transferase (GST) was assayed by the method of Habig et al (1974).

2.12.1 Chemicals and Reagents

1. 0.2 M Phosphate buffer (pH 6.5): 6.8 ml of 0.2 M solution of monobasic sodium phosphate was mixed with 31.5 ml of 0.2 M solution of dibasic sodium phosphate, pH was adjusted to 6.5 and diluted to a total of 200 ml with DDW.
2. 1 mM Glutathione: 30 mg of glutathione (reduced) was dissolved in 100 ml of phosphate buffer (pH 6.5).
3. Substrate: 1mM CDNB was prepared by dissolving 30 mg of CDNB (1-chloro-2, 4-dinitrobenzene) in 1 ml acetone.
4. Acetone: Analytical Grade

2.12.2 Isolation of Post-Mitochondrial Fraction (PMF): Different regions of the CNS were homogenized in chilled phosphate buffer (10% w/v) and centrifuged in cool for 15 min at 15000 rpm.

2.12.3 Assay of GST: In 0.1 ml of post-mitochondrial fraction (supernatant), 2.7 ml glutathione solution and 0.2 ml substrate were mixed. The optical density (OD) was recorded at 340 nm against blank at every 15 sec for 3 min. The blank contained 0.1 ml DDW in place of PMF.

Calculation:

$$\text{GST Activity} = \frac{\text{OD} \times 625 \text{ (factor)}}{\text{Protein conc. (mg)}} \text{ units/mg protein}$$

where OD = change of OD per minute

2.12.4 Activity units: A unit of activity is defined as the amount of enzyme catalyzing the formation of 1 mole of CDNB conjugate per minute under specific assay conditions. Specific activity is defined as the units of enzyme activity per mg of protein, as measured by the method of Lowry et al (1951), with bovine serum albumin as standard.

2.13 Superoxide dismutase :

Superoxide dismutase (SOD) activity was measured following the procedure of Marklund & Manrklund (1974).

The principle of the procedure depends upon autoxidation of pyrogallol.

$$\text{Pyrogallol} + O_2 \xrightarrow{\text{autoxidation}} \text{Oxidation product} + O_2$$

$$2\,O_2 + 2\,H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2$$

2.13.1 Chemicals and Reagents:

1. 0.05 M Tris-Succinate buffer (pH 8.2): Dissolve 606 mg Tris in approx. 60 ml DDW. To this, 39.3 mg EDTA was added and the pH was adjusted to 8.2 with succinic acid solution. The volume was then made up to 100 ml with DDW.

2. Succinic acid solution, 0.05 M: 590 mg succinic acid was dissolved in 100 ml DDW.
3. Pyrogallol solution, 8 mM: 100 mg pyrogallol was dissolved in 100 ml DDW. This solution was prepared fresh on the day of experiment.

2.13.2 Procedure: Different saline cleaned CNS parts were homogenized (10% w/v) in chilled 0.15 M KCl and centrifuged in cool at 10,000 rpm for 15 min. The supernatant was used as sample solution. 0.05 ml of the sample solution was added to 2.85 ml tris-succinate buffer, mixed well and incubated at 25°C for 20 min. The reaction was started by adding 0.1 ml pyrogallol solution. The contents were shaken well and change in absorbance per min was immediately recorded for 3 minutes at 420 nm on Beckman DU-6 UV/vis spectrophotometer. A reference set, consisting of 0.05 ml DDW instead of the sample solution, was also run similarly.

Calculation:

$$\text{SOD Activity} = \frac{(A/min_{ref} - A/min\ sample) \times 30}{A/min_{ref} \times 0.05 \times 1} \text{ units/10 mg tissue}$$

where A/min_{ref} = change of absorbance per min in reference set

A/min_{sample} = change of absorbance per min in sample

2.13.3 Activity Unit: One unit of the enzyme is defined as the amount of enzyme which causes a 50% inhibition of pyrogallol autoxidation under assay conditions.

2.14 Nucleic Acids

2.14.1 Isolation of Nucleic Acids:

Nucleic acids were isolated following the method of Searchy and MacInnis (1970). Weighed tissue of the different CNS regions were homogenized in 5.0 ml of 0.5 N perchloric acid. The homogenate was heated at 90°C in boiling water bath for 10 min, cooled and centrifuged at 3,000 rpm for 10 min. The supernatant was taken in graduated test tubes and the volume was maintained upto 5.0 ml with 0.5 N perchloric acid. This extract was then used in the estimation of DNA and RNA according to the following procedures.

2.14.2 Estimation of DNA:

DNA was estimated following the method of Burton (1956).

2.14.2.1 Chemicals and Reagents:

1. Diphenylamine Reagent: 1.5 g diphenylamine was dissolved in about 50-60 ml glacial acetic acid. 1.5 ml conc, H_2SO_4 was added to it and the final volume was maintained upton 100 ml.

2.14.2.2 Procedure: 2.0 ml of the supernatant of nucleic acid extract was taken in a test tube. To this, 4.0 ml diphenylamine reagent was added, and the tubes were heated on boiling water bath for 15 min, After cooling, the colour intensity was measured at 600 nm against a reagent blank.

2.14.3 Estimation of RNA:

RNA was estimated of Dische (1955).

2.14.3.1 Chemicals and Reagents:

1. Oricinol Reagent: 33.0 mg ferric chloride was dissolved in about 50 ml conc. HCl, 3.5 ml 6% orcinol (dissolved in absolute alcohol) was mixed with it, and the volume was made upto 100 ml with HCl.

2.14.3.2 Procedure: 2.0 ml of the supernatant was taken in test tubes. 4.0 ml of the orcinol reagent was added to it. Test tubes were heated on boiling water bath for 15 min, cooled and the absorbance was read at 660 nm against a reagent blank.

Calculation:

Both RNA and DNA were calculated by the following formula:

$$\text{RNA or DNA (mg/g fresh tissue wt)} = \frac{C \times V}{Vt \times Wt}$$

where C = Concentration in g in 2.0 ml extract

Vt = Volume taken for the estimation

V = Total volume of the extract (4 ml)

Wt = Fresh weight of the tissue in milligram.

2.15 Estimation of Protein:

Protein was analysed following the well known method of Lowry et al. (1951). Reagents:

2.15.1 Chemicals and Reagents

1. Standard solution: A standard solution of 1.0 mg BSA/ml was prepared in PBS.
2. Copper Reagent:
   Reagent A : 4% sodium carbonate in DDW
   Reagent B : 2% copper sulphate in DDW
   Reagent C : 4% sodium-potassium tartarate in DDW

   Copper reagent was prepared by mixing reagents A, B and C in a ratio of 100:1:1 respectively at the time of use.
3. Folin-Ciocaltau Reagent: Obtained from Patel Chest Institute, New Delhi.

2.15.2 Procedure: Residue left in the test ubes after taking the supernatant for nucleic acid estimation, was dissolved in 5.0 ml DDW. 0.1 ml of the aliquot was taken in the test tubes from this solution and the volume was maintained upto 1.0 ml with DDW. To this, 5.0 ml of copper reagent was added and the tubes were shaken thoroughly on a cyclo-mixer. After 10 min, 1.0 ml of the Follin's reagent (diluted 5 times) was added. The intensity of colour was measured at 700 nm after 30 min, against a reagent blank. A standard curve of different concentrations of BSA (100-600 μg) was plotted in the same way as described above.

2.16 MICROANALYSIS OF DIMECRON BY THIN-LAYER CHROMATOGRAPHY:

The dimecron residue was detected from the central nervous system by thin-layer chromatography, and subsequently analysed spectrophotometrically using phosphomolybdenum method, following the procerdure of Anliker and Menzer (1963), modified in this laboratory by Naqvi and Hasan (1990).

2.16.1 Animals: Twentyfour male albino rats of Charles Foster strain, weighing 150 ± 20 g were obtained from Central Animal House of J.N. Medical College, A.M.U., Aligarh, and allowed free access to pellet diet and water.

2.16.2 Experimental Design: Animals were divided into four groups of six each. Freshly prepared doses (1.0, 1.5 and 2.0 mg/Kg b. wt) of dimecron were administered intraperitoneally to the animals of three groups, respectively, for a period of seven days. The vehicle used for the dose was physiological saline. The fourth group, used as control, received equal volume of physiological saline concurrently.

On the seventh day all the rats were sacrificed by cervical dislocation followed by decapitation. Brains were removed and cerebrum, cerebellum, brain stem and spinal cord were dissected out rapidly on ice-plate and weighed to the nearest milligram.

2.16.3 Chemicals and Reagents:

1. Methylene chloride (A.R.)
2. Hydrogen peroxide, 30% (A.R.)
3. Chromatography solvent: 5 parts Petroleum ether (b.p. 90° - 100°C), 5 parts Toluene, 7 parts Methanol, and 3 parts DDW were mixed well and the two phases were separated.
4. Blue tetrazolium solution: 3,3-dimethoxy-4, 4-biphenylene) -bis (2,5-diphenyl-2 H-tetrazolium chloride), 0.1%
5. Potassium permanganate solution, 0.01 N.
6. Sodium oxalate solution, 0.01 N.
7. Ammonium molybdate solution, 5%
8. ANSA solution: 0.2% 1-amino-2-naphthol-4-sulfonic acid was mixed with 12% sodium metabisulfite and 1.2% anhydrous sodium sulfite.
9. Conc. Sulphuric acid. (A.R.)
10. Sodium hydroxide, 2 N. (A.R.)
11. n-Butanol (A.R.)

2.16.4 Sample preparation: Saline-cleaned and accurately weighed CNS parts were homogenized (10% w/v) in methylene chloride and centrifuged at 3000 rpm for 20 min. The supernatnat was evaporated to dryness and the residue left was dissolved in 2.0 ml methylene chloride.

2.16.5 Thin-Layer Chromatography: 20 x 20 cm glass plates were coated with 0.25 mm silica gel G and dried in over for 2 hrs. Thereafter, these plates were conditioned by running the TLC in ascending order with the upper phase of the chromatography solvent. After drying in air, they are were

again subjected to TLC with the lower phase. This conditioning of TLC plates was done just prior to use.

From the sample of each CNS region, 50 µl aliquot was applied one centimeter above the base of pre-conditioned TLC plates. The plates were then placed in the chromatography jar and thin-layer chromatography was run with upper phase of chromatography solvent, and dried in air. The detector (0.1% blue tetrazolium solution - 2 NaOH, 1:9 v/v) was then sprayed on the plates leading to the appearance of blue spots of dimecron (phosphamidon) and its metabolite desethylphosphamidon (Fig 3.9).

Rf values of the spots were measured, and the spots scrapped in the test tubes, 2.0 ml of methanol was added to it and left for overnight.

2.16.6 Estimation of Dimecron:

The tubes were then centrifuged at 5,000 rpm for 10 min. To the filterate, 0.5 ml conc. sulphuric acid and one glass beed were added, and the methanol was boiled off on the boiling water bath. For decolorization, 2-3 drops of H_2O_2 were added, and the tubes were again placed on the boiling water bath for 5 minutes or until a colourless solution was obtained. Tubes were heated for an extra 20 min and then cooled in ice. To this, 5.0 ml of DDW as mixed, followed by dropwise addition of $KMnO_4$ until a pink colour persisted. One drop of sodium oxalate solution was added and the pink

colour disappeared. Thereafter, the tubes were heated on boiling water bath for 20 min. After cooling, 5.0 ml DDW, 0.5 ml ammonium molybdate solution, and 0.5 ml ANSA solution, were added and again heated on water bath for 20 min, to get a blue coloured solution. After cooling in ice, 4.0 ml n-butanol was mixed while shaking. Two phases were allowed to separate. The upper layer was taken in cuvette and the optical density was recorded at 790 nm against standard of n-butanol.

2.16.7 Preparation of standard curve: The whole process of thin-layer chromatography was similarly carried out as described earlier, by applying 10, 20, 30, 40, 50, 60 ul of dimecron on the TLC plates, instead of brain aliquots, and Rf value of the standard dimecron was measured. This was followed by preparation of standard curve using spectrophotometric estimation of dimecron as described earlier in the microanalysis of dimecron in the brain samples.

Calculation:

$$\text{Concentration of Dimecron (mg/g tissue)} = \frac{C \times V}{Vt \times Wt}$$

where C= Concentration of dimecron in volume taken, (OD x factor)

V= Total volume of homogenate supernatant

Vt= Volume applied on the TLC plate

Wt= Fresh weight of the tissue (in g)

2.17 Statistical Analysis:

Results were statistically analysed using 'two-sampled' student's 't' test. 'P' values less than 0.05 were considered to be significant.

Moreover, in the Microanalysis of Dimecron, the Analysis of Variance (ANOVA) was used for statistical analysis of the variations in the accumulation of dimecron between various CNS regions and between different doses of dimecron.

3. RESULTS

3.1.0 Physical Signs After Dimecron Intoxication

Dimecron intoxication (2.0 mg/Kg b.wt, ip x 7 days) was found to produce physical signs such as muscular fasciculations, convulsions and ataxia. After 5-6 days of injections, all the treated rats became lethargic. Loss of weight of the order of 30-40g, was also noticed on the seventh day.

3.2.0 Open Field Behaviour:

3.2.1 Effect of Dimecron on Open Field Behaviour (OFB):

A gradual daily decrease was observed in the open field behaviour parameters, i.e. ambulation (Table 3.1), rearing (Table 3.2) and rearing (Table 3.3) of the rats treated with dimecron (2.0 mg/ kg b.wt. ip) daily for seven days.

Ambulation and preening scores showed significant decrement ($P<0.001$) from the third day of injections onwards. Rearing score, however, was reported to decrease significantly ($P<0.001$) from the fifth day.

On the seventh and last day, maximum decrease was noticed in the rearing score (-95%) as compared to ambulation (-89%) and preening (-70%) scores.

TABLE 3.1

Perturbation in the ambulation score of the rats treated with dimecron for 7 days: Protection by cithilone

(Data are mean ± SE of daily 3 observations of six animals)

Control	Days	Experimental I		Experimental II	
		Mean ± SE	% change**	Mean ± SE	% change
20.66 ± 0.78	Day O	19.79 ± 0.06	NS	19.09 ± 0.22	NS
	Day 1	19.83 ± 0.05	NS	19.12 ± 0.09	NS
	Day 3	17.50 ± 0.47*	-15	19.33 ± 0.12	NS
	Day 5	11.50 ± 0.61*	-44	19.26 ± 0.54	NS
	Day 7	2.23 ± 0.36*	-89	19.18 ± 0.48	NS

With respect to control value: * P < 0.001; ** Percent alteration; NS = Not significant
Day O : Pre-injection observation of treated rats.
Exp I : Dimecron (2 mg / kg b. wt, ip);
Exp II : Dimecron + Cithiolone (8 mg/ Kg b. wt, ip).

Fig.3·1 AMBULATION SCORE OF THE RAT TREATED WITH DIMECRON : PROTECTION BY CITHIOLONE.

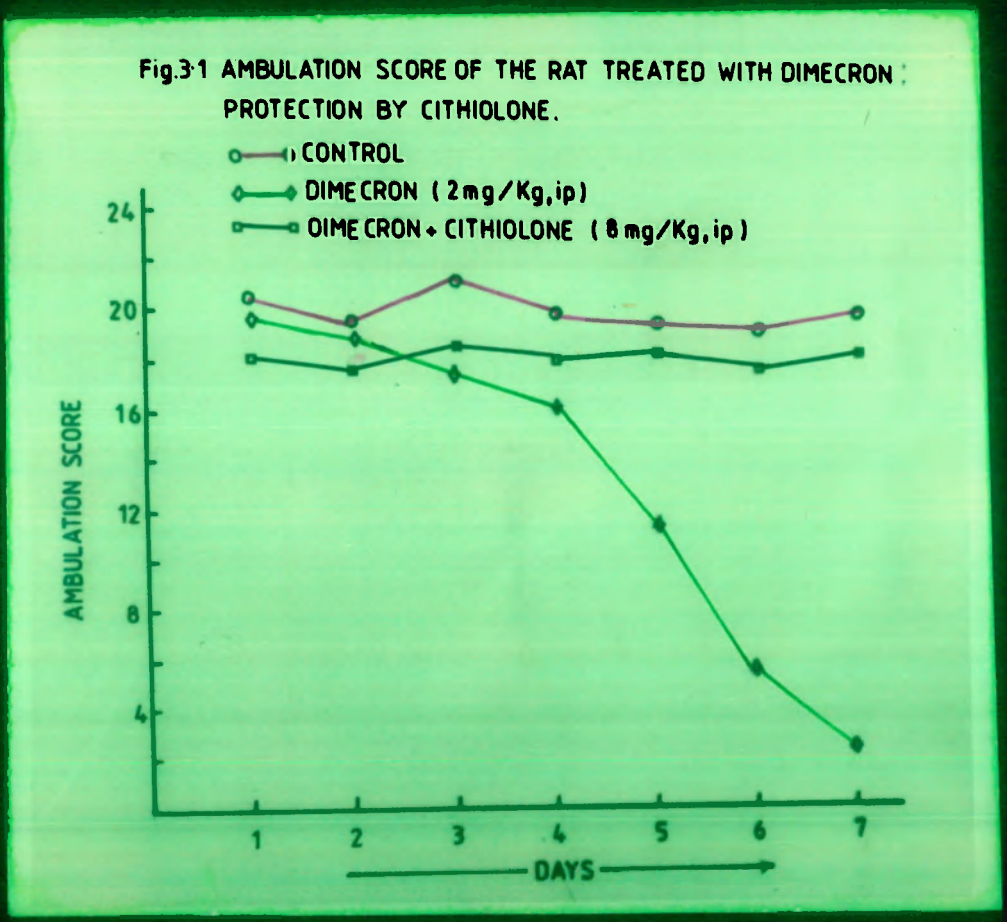


TABLE 3.2

Perturbation in the rearing score of the rats treated with dimecron for 7 days: Protection by cithilone

(Data are mean ± SE of daily 3 observations of six animals)

Control	Days	Experimental I		Experimental II	
		Mean ± SE	% change**	Mean ± SE	% change
10.30 ± 1.05	Day 0	10.81 ± 0.89	NS	10.44 ± 0.95	NS
	Day 1	10.66 ± 0.36	NS	10.41 ± 1.02	NS
	Day 3	8.66 ± 0.36	NS	10.53 ± 1.13	NS
	Day 5	3.83 ± 0.36*	-63	10.21 ± 1.19	NS
	Day 7	0.50 ± 0.24*	-95	9.88 ± 0.82	NS

With respect to control value: * P < 0.001; ** Percent alteration; NS = Not significant
Day 0 : Pre-injection observation of treated rats.
Exp I : Dimecron (2 mg / kg b. wt, ip);
Exp II : Dimecron + Cithiolone (8 mg/ Kg b. wt, ip).

Fig 3.2 REARING SCORE OF DIMECRON TREATED RATS: PROTECTION BY CITHIOLONE

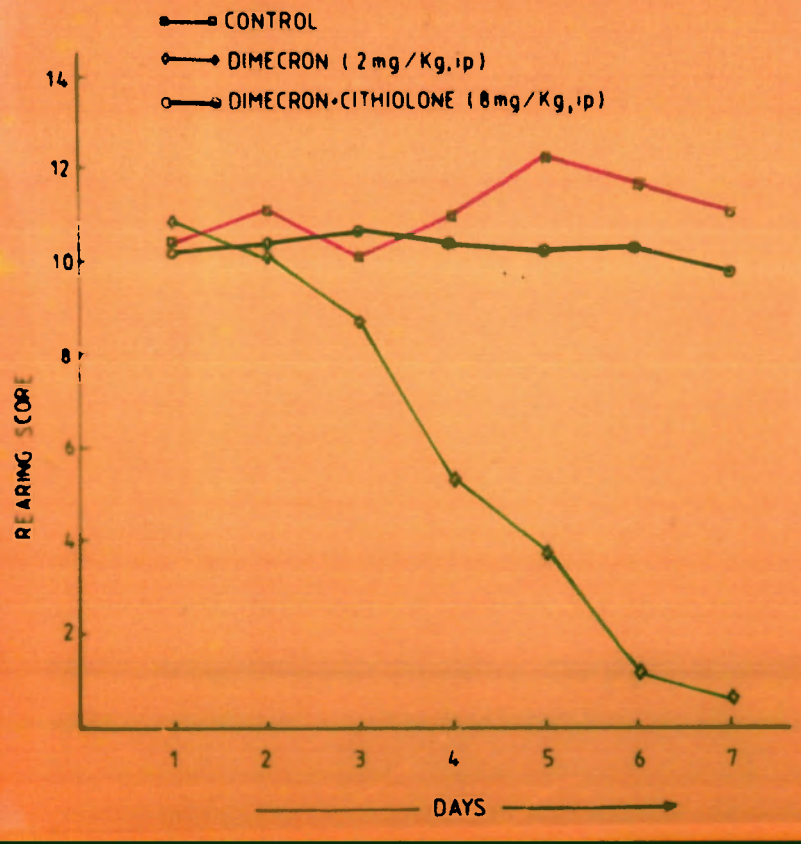


TABLE 3.3

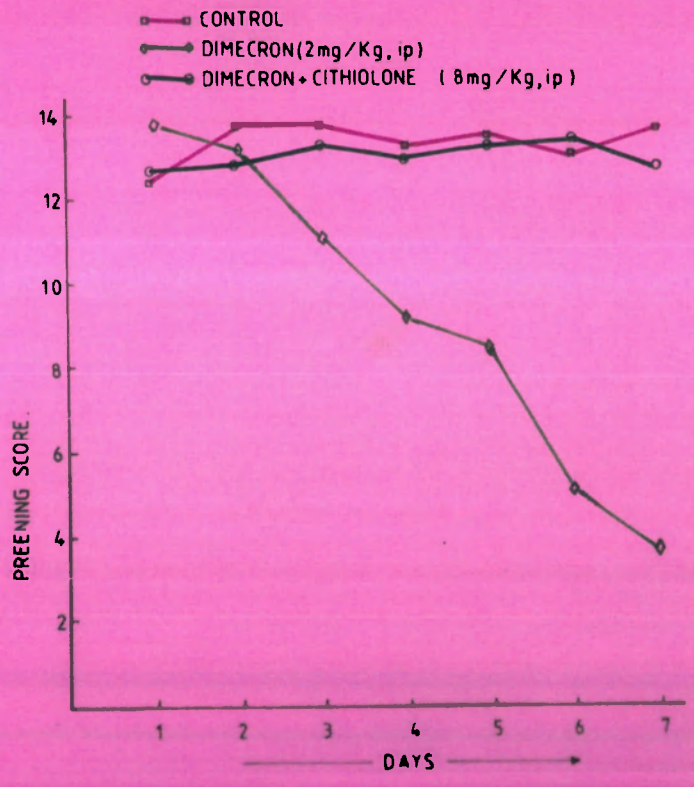
Perturbation in the preening score of the rats treated with dimecron for 7 days: Protection by cithilone

(Data are mean ± SE of daily 3 observations of six animals)

Control	Days	Experimental I		Experimental II	
		Mean ± SE	% change**	Mean ± SE	% change
12.50 ± 0.83	Day 0	13.75 ± 0.93	NS	12.89 ± 0.81	NS
	Day 1	13.83 ± 0.52	NS	12.51 ± 0.49	NS
	Day 3	11.00 ± 0.40*	-12	13.28 ± 0.63	NS
	Day 5	8.50 ± 0.47*	-32	13.44 ± 0.95	NS
	Day 7	3.66 ± 0.36*	-70	12.76 ± 0.47	NS

With respect to control value: * $P < 0.001$; ** Percent alteration; NS = Not significant
Day 0 : Pre-injection observation of treated rats.
Exp I : Dimecron (2 mg / kg b. wt, ip);
Exp II : Dimecron + Cithiolone (8 mg/ Kg b. wt, ip).

Fig.3·3 PREENING SCORE OF THE RATS TREATED WITH DIMECRON : PROTECTION BY CITHIOLONE



3.2.2 Protective Effect of Cithiolone Against the Dimecron-induced Perturbation of OFB:

Interestingly, when the rats were treated with cithiolone (8.0 mg/kg b.wt,ip) simultaneously with dimecron, no remarkable change was seen in the open field behaviour, and ambulation (Table 3.1), rearing (Table 3.2) and preening (Table 3.3) scores were observed to be near the normal (control) values throughout the seven days of experiment. Figures 3.1, 3.2 and 3.3 clearly depict the protection by cithiolone against the effects of dimecron on the ambulation, rearing and preening, respectively.

3.3.0 Acetylcholinesterase

3.3.1 Effect of Dimecron on Acetylcholinesterase:

Dimecron toxicosis (2.0 mg/kg b. wt. ip x 7 days) was found to induce significant inhibition of acetylcholinesterase (AChE) activity (Table 3.4). Inhibition of AChE was reported in cerebrum, brain stem, spinal cord ($P<0.001$) and cerebellum ($P<0.01$). Spinal cord showed maximum AChE depletion (-27%).

3.3.2 Protective Effect of Cithiolone Against Dimecron-induced AChE inhibition

Simultaneous treatment of cithiolone (8.0 mg/kg wt, ip) alongwith dimecron was reported to protect remarkably against

TABLE 3.4

Inhibition of AChE activity in various regions of the CNS following dimecron intoxication (2.0 mg / Kg b. wt, ip x 7 days)

(Values,expressed as u moles of acetylthiocholine iodide hydrolysed per gram of tissue per minute, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	2.84 ± 0.016	2.29 ± 0.020**	-19
Cerebellum	1.28 ± 0.026	0.96 ± 0.024*	-25
Brain stem	3.34 ± 0.030	2.48 ± 0.045**	-25
Spinal cord	2.26 ± 0.040	1.64 ± 0.030**	-27

Degree of significance: P: * <0.01, ** <0.001

TABLE 3.5

Protective effect of cithiolone on dimecron-induced alteration of acetylcholinesterase activity in various CNS regions.

(Data expressed as u moles of acetylthiocholine iodide hydrolysed per gram of tissue per minute, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	2.84 ± 0.016	2.80 ± 0.024	NS
Cerebellum	1.28 ± 0.026	1.22 ± 0.027	NS
Brain stem	3.34 ± 0.030	3.33 ± 0.022	NS
Spinal cord	2.26 ± 0.040	2.27 ± 0.030	NS

Experimental: Cithiolone (8.0 mg/Kg) + Dimecron (2.0 mg /Kg b. wt, ip x 7 days)
NS : Not significant

TABLE 3.6

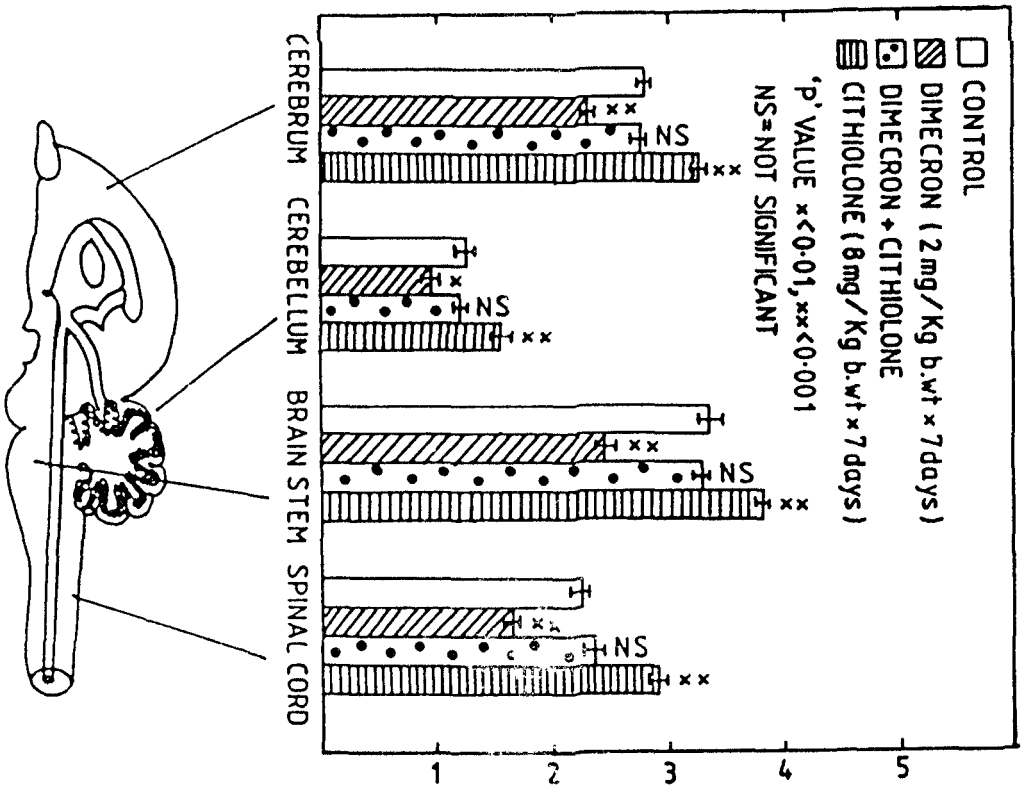
Effect of cithiolone on the activity of acetylcholinesterase in the CNS regions

(Values expressed as u moles of acetylthiocholine iodide hydrolysed per gram of tissue per minute, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	2.84 ± 0.016	3.26 ± 0.012*	14
Cerebellum	1.28 ± 0.026	1.52 ± 0.014*	18
Brain stem	3.34 ± 0.030	3.87 ± 0.015*	15
Spinal cord	2.26 ± 0.040	2.88 ± 0.015*	27

Experimental: Cithiolone (8.0 mg/Kg b.wt, ip x 7 days)
Degree of significance: P: * <0.001

FIG. 3·4 : DIMECRON INDUCED ALTERATION OF AChE ACTIVITY: PROTECTION BY CITHIOLONE.



dimecron induced depletion of AChE activity in all the CNS regions (Table 3.5).

Notably, when rats were exposed to cithiolone alone (Table 3.6), significant elevation of AChE activity ($P<0.001$) was seen in the cerebrun, cerebellum, brain stem and spinal cord, with the maximum elevation being noted in the spinal cord.

Figure 3.4 clearly depicts effect of dimecron on the AchE: Correlated with protection by cithiolone.

3.4.0. Effect of Dimecron on Brain Monoamines

Level of monoamines was reported to deplete significantly in all the CNS regions of the rats intoxicated with dimecron (2.0 mg / Kg b.wt. ip) daily for seven days.

Dopamine (DA) was found to decrease (Table 3.7) in cerebrum, cerebellum, brain stem ($P<0.010$) and spinal cord ($P<0.001$). Cerebellum and brain stem showed the maximum (-24%) and minimum (-14%) decrement, respectively. Table 3.8 portrays the diminished contents of norepinephrine (NE) in cerebrum ($P<0.01$), cerebellum ($P<0.05$), brain stem ($P<0.001$) and spinal cord ($P<0.02$), with the greatest depletion found in the brain stem (-21%). Serotonin (5-HT) also showed depletion (Table 3.9) in cerebrum, spinal cord ($P<0.01$), cerebellum ($P<0.02$) and brain stem ($P<0.001$). Maximum decrease was found in cerebrum (-22%).

TABLE 3.7

Alteration in the level of dopamine in various CNS regions of rats treated with dimecron (2.0 mg / Kg b. wt, ip x 7 days)

(Data, expressed as Mg/g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	0.48 ± 0.005	0.40 ± 0.010*	-16
Cerebellum	0.35 ± 0.012	0.26 ± 0.009*	-24
Brain stem	0.38 ± 0.020	0.32 ± 0.004*	-14
Spinal cord	0.27 ± 0.007	0.21 ± 0.002**	-21

Degree of significance: P: * <0.01, ** <0.001

TABLE 3.8

Alteration in the level of norepinephrine in various CNS regions of rats treated with dimecron (2.0 mg / Kg b. wt, ip x 7 days)

(Data, expressed as ug/g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	1.62 ± 0.042	1.36 ± 0.041**	-16
Cerebellum	1.30 ± 0.037	1.07 ± 0.045+	-18
Brain stem	1.12 ± 0.059	0.88 ± 0.033***	-21
Spinal cord	0.87 ± 0.030	0.71 ± 0.040*	-18

Degree of significane: P: + <0.05 ; * <0.02, ** <0.01, *** <0.001

TABLE 3.9

Alteration in the level of 5 - HT in various CNS regions of rats treated with dimecron (2.0 mg / Kg b. wt, ip x 7 days)

(Data, expressed as Mg / g fresh tissue, are mean SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	0.39 ± 0.022	0.29 ± 0.01****	-22
Cerebellum	0.32 ± 0.014	0.25 ± 0.016*	-21
Brain stem	0.42 ± 0.015	0.33 ± 0.012***	-22
Spinal cord	0.30 ± 0.010	0.25 ± 0.008**	-17

Degree of significance: P< * 0.02, ** 0.01, *** 0.001

3.5.0 Monoamine Oxidase (MAO) in Brain

3.5.1 Effect of Dimecron on MAO Activity

Following dimecron administration (2.0 mg/kg b.wt. ip x 7 days), the activity of monoamine oxidase was significantly ($P<0.001$) enhanced in all the regions of the CNS.

Elevation of the MAO activity was found of the order of 21% in cerebrum, 24% in cerebellum, 19% in brain stem and 23% in spinal cord (Table 3.10). Notably, cerebellum and brain stem showed maximum and minimum increment, respectively.

3.5.2. Protective Effect of Cithiolone on the Dimecron-induced MAO Elevation

Interestingly, a remarkable protection against the effect of dimecron was observed on the MAO activity, when the rats were treated with cithiolone (8.0 mg / kg b.wt,ip) simultaneously with dimecron (Table 3.11). No significant change as compared to the control values, was observed in the MAO activity of all the CNS regions.

When cithiolone was administered alone, however, significant inhibition ($P<0.001$) was noted in the activity of MAO in all the regions, with the maximum and minimum depletion, being observed in cerebellum (-28%) and brain stem (-21%), respectively (Table 3.12). Protective effect of cithilone on the MAO activity is shown in Fig. 3.5

TABLE 3.10

Elevation of monoamine oxidase activity in various CNS regions following dimecron intoxication (2.0 mg / Kg b. wt, ip x 7 days)

(Values, expressed as u moles of benzaldehyde formed/min/mg protein, are mean ± SE of 6 animals)

CNS regions	Control	Experimental	% change
Cerebrum	4.74 ± 0.091	5.76 ± 0.025*	21
Cerebellum	5.75 ± 0.036	7.15 ± 0.017*	24
Brain stem	5.17 ± 0.016	6.17 ± 0.016*	19
Spinal cord	6.05 ± 0.026	7.46 ± 0.042*	23

P value: * < 0.001

TABLE 3.11

Protective effect of cithiolone on dimecron -induced perturbations in the monoamine oxidase activity in CNS regions.

(Data, expressed as u moles of benzaldehyde formed/min/mg/protein, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	4.74 ± 0.091	4.77 ± 0.046	NS
Cerebellum	5.75 ± 0.036	5.79 ± 0.050	NS
Brain stem	5.17 ± 0.016	5.13 ± 0.038	NS
Spinal cord	6.05 ± 0.026	5.99 ± 0.040	NS

Experimental: Cithiolone (8.0 mg/Kg) + Dimecron (2.0 mg/Kg b. wt) ip x 7 days NS = Not significant

TABLE 3.12

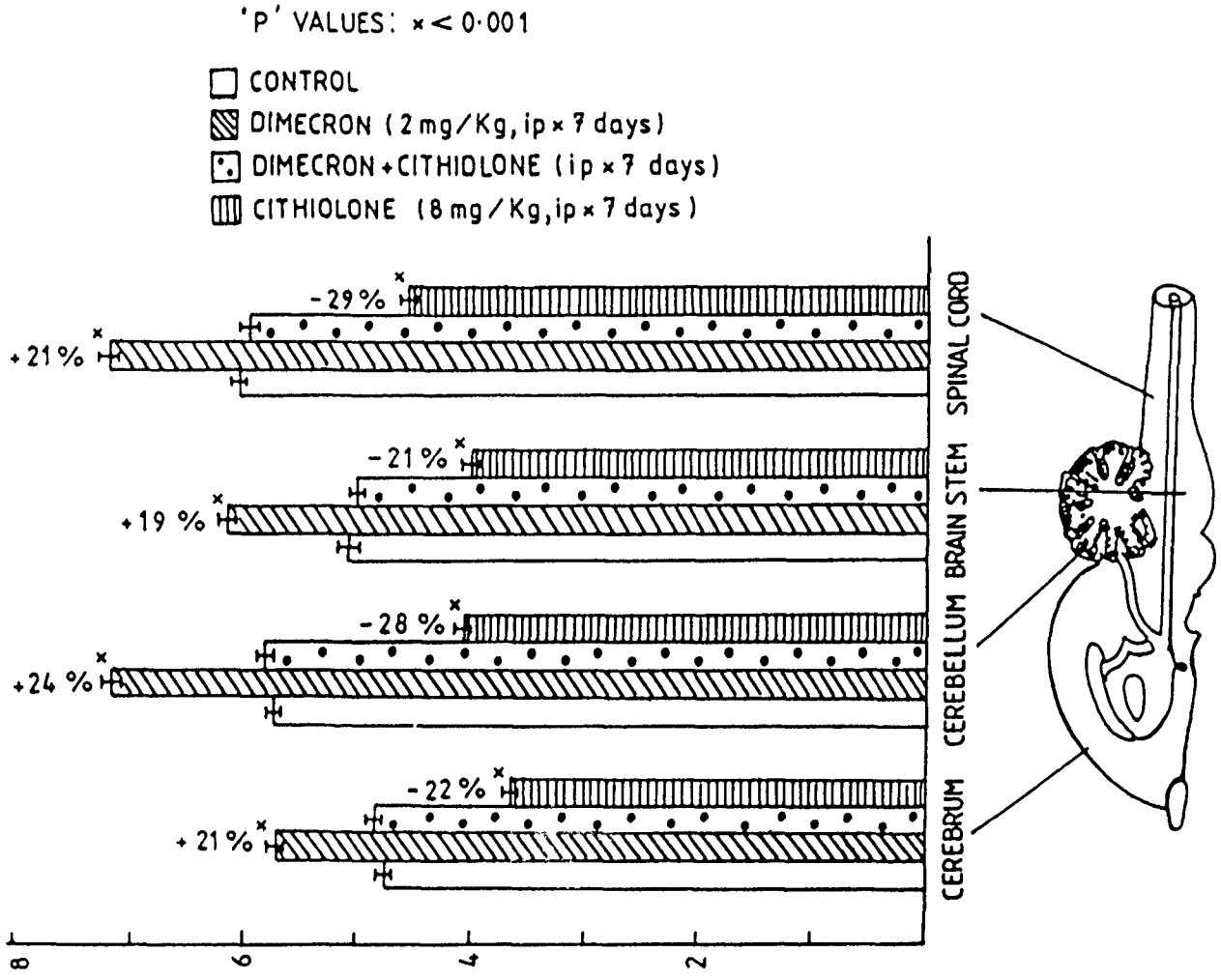
Effect of cithiolone on the activity of monoamine oxidase in various regions of the CNS.

(Values, expressed as u mole of benzaldehyde formed/ min/ mg protein, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	4.74 ± 0.091	3.68 ± 0.107*	-22
Cerebellum	5.75 ± 0.036	4.13 ± 0.038*	-28
Brain stem	5.17 ± 0.016	4.07 ± 0.074*	-21
Spinal cord	6.05 ± 0.026	4.59 ± 0.021*	-24

Experimental: Cithiolone (8.0 mg / Kg b. wt, ip x 7 days)
Degree of significance: P* < 0.001

Fig. 3·5. DIMECRON INDUCED ALTERATION IN MAO ACTIVITY: PROTECTION BY CITHIOLONE.



3.6.0. Effect of Dimecron on Brain Lipids

Effects of dimecron (2.0 mg/kg b. wt. ip x 7 days) were studied on the concentration of total lipids, phospholipids, cholesterol and gangliosides in various regions of the CNS.

3.6.1. Total Lipids

Table 3.13 describes the alterations in the concentration of total lipids in various CNS regions following dimecron toxicity.

Total lipids were found to decrease considerably in cerebrum ($P<0.001$), cerebellum, brain stem ($P<0.01$) and spinal cord ($P<0.05$), with the most significant change (-21%), observed in cerebrum.

3.6.2. Phospholipids

Phospholipid contents diminished significantly ($P<0.001$) in all the CNS regions after dimecron toxicosis (Table 3.14). Cerebrum showed maximum (-57%) depletion, while the minimum depletion was found in brain stem (-29%). Cerebellum and spinal cord revealed decrement of phospholipids of the order of -46% and -36%, respectively.

3.6.3. Cholesterol

Dimecron intoxication led to remarkable decrement in the level of cholesterol (Table 3.15) in the cerebrum, cerebellum ($P<0.001$) and spinal cord ($P<0.01$). Maximum

TABLE 3.13

Alteration of total lipids in different regions of the rat CNS, following the administration of dimecron (2.0 mg / Kg b. wt, ip x 7 days)

(Values, expressed as mg/ g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	154.64 ± 6.19	121.99 ± 3.19***	-21
Cerebellum	128.78 ± 3.16	114.57 ± 3.93**	-21
Brain stem	162.42 ± 10.68	136.14 ± 6.92**	-16
Spinal cord	237.60 ± 7.32	206.03 ± 10.58*	-13

Degree of significance : P < * 0.05, ** 0.01, *** 0.001

TABLE 3.14

Alteration of phospholipids in different regions of the rat CNS, following the administration of dimecron (2.0 mg / Kg b. wt, ip x 7 days)

(Values, expressed as mg/ g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	55.45 ± 0.07	23.86 ± 2.19*	-57
Cerebellum	53.90 ± 0.36	29.24 ± 2.33*	-46
Brain stem	73.81 ± 1.57	52.81 ± 7.73*	-29
Spinal cord	77.60 ± 0.89	49.89 ± 7.19*	-36

Degree of significance : P <* 0.001

TABLE 3.15

Alteration of cholesterol in different regions of the rat CNS, following the administration of dimecron (2.0 mg / Kg b. wt, ip x 7 days)

(Values, expressed as mg/ g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	29.70 ± 6.19	19.36 ± 1.00**	-35
Cerebellum	24.11 ± 3.16	16.91 ± 1.50**	-29
Brain stem	31.34 ± 10.68	29.80 ± 3.64^{NS}	-16
Spinal cord	43.12 ± 7.32	37.55 ± 4.20*	-12

NS = Not Significant
Degree of significance : P < * 0.01, ** 0.001

TABLE 3.16

Alteration of Gangliosides in different regions of the rat CNS, following the administration of dimecron (2.0 mg / Kg b. wt, ip) for 7 days)

(Values, expressed as mg/ g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	1.17 ± 0.08	0.34 ± 7.74*	-71
Cerebellum	1.10 ± 0.02	0.29 ± 0.03*	-73
Brain stem	0.50 ± 0.06	0.13 ± 0.03*	-74
Spinal cord	0.31 ± 0.01	0.27 ± 0.06	-13

(Values, expressed as mg/ g fresh tissue, are mean ± SE of six animals)
NS = Not Significant
Degree of significance : P < * 0.001

decrease was reported in the cerebrum (-35%) while a non-significant change was seen in the brain stem (-5%).

3.6.4. Gangliosides:

The concentration of total gangliosides was found to diminish significantly ($P<0.001$) in cerebrum, cerebellum and brain stem (Table 3.16) following dimecron toxicosis. In the spinal cord, however, the change was not significant (-13%).

3.7.0. Lipid Peroxidation in Brain

3.7.1. Effect of Dimecron on Lipid Peroxidation:

The rate of lipid peroxidation was reported to enhance significantly in various regions of the CNS following intoxication with dimecron (2.0 mg / kg b. wt, ip) daily for seven days (Table 3.17).

Significant increment was observed in cerebellum ($P<0.02$), brain stem and spinal cord ($P<0.001$).

3.7.2. Protective Effect of Cithiolone Against Dimecron-induced Elevation of Lipid Peroxidation:

Interestingly, when cithiolone (8.0 mg/kg b.wt, ip) was administered simultaneously with dimecron, a remarkable protection against elevation of the rate of lipid peroxidation was obse[illegible] (Fig. 3.6). The rate of lipid peroxidation was reported to be near the control values in

TABLE 3.17

Perturbations in the rate of lipid peroxidation in different regions of the rat CNS, following the administration of dimecron (2.0 mg / Kg b. wt, ip x 7 days).

(Values, expressed as nanomoles of malonaldialdehyde / 30 min, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	10.14 ± 0.88	9.06 ± 0.43	NS
Cerebellum	7.30 ± 0.35	8.92 ± 0.70*	+22
Brain stem	3.11 ± 0.16	4.38 ± 0.18**	+40
Spinal cord	1.50 ± 0.15	2.42 ± 0.13**	+61

NS = Not Significant
Degree of significance : P < * 0.02, ** 0.001

TABLE 3.18

Protective effect of cithiolone on dimecron-induced alterations in the rate of lipid peroxidation in CNS regions

(Data, expressed as n moles of malonaldialdehyde formed / 30 min, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	10.74 ± 0.88	9.63 ± 0.08	NS
Cerebellum	7.30 ± 0.35	7.52 ± 0.31	NS
Brain stem	3.11 ± 0.16	3.42 ± 0.07	NS
Spinal cord	1.50 ± 0.15	1.58 ± 0.05	NS

Experimental: Cithiolone (8.0 mg/Kg) + Dimecron (2.0 mg / Kg b. wt, ip x 7 days)
NS = Not significant

TABLE 3.19

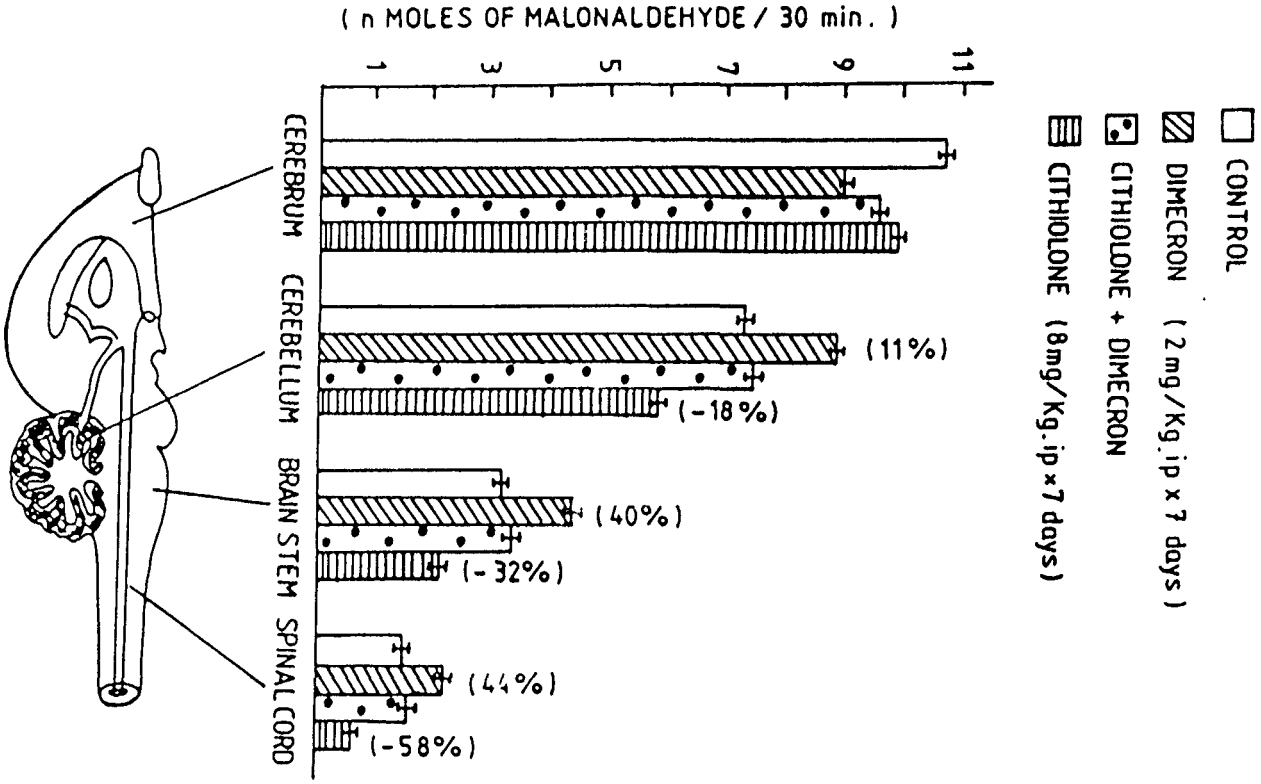
Effect of cithiolone on the rate of lipid peroxidation in various regions of the CNS.

(Data, expressed as moles of malonaldialdehyde formed / 30 min, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	10.74 ± 0.88	9.88 ± 0.01	NS
Cerebellum	7.30 ± 0.35	5.97 ± 0.05*	-18
Brain stem	3.11 ± 0.16	2.11 ± 0.01**	-32
Spinal cord	1.50 ± 0.15	0.63 ± 0.01	-58

Experimental: Cithiolone (8.0 mg/Kg b. wt, ip x 7 days)
Degree of significance: * P< 0.01, ** P <0.001,
NS : Not significant

Fig.3·6. DIMECRON INDUCED ALTERATION IN RATE OF LIPID PEROXIDATION : PROTECTION BY CITHIOLONE.



cerebrum, cerebellum, brain stem and spinal cord (Table 3.18).

Significant depletion in the rate of lipid peroxidation, however, was observed in cerebellum ($P<0.01$), brain stem and spinal cord ($P<0.001$), when the rats were exposed to cithiolone alone (Table 3.19).

3.8.0 Effect of Dimecron on Sulfhydryl Groups in Brain

Alterations in the content of total, non-protein bound (NPB) and protein-bound sulfhydryl (-SH) groups in various CNS regions of the dimecron (2.0 mg / kg b. wt. ip x 7 days) treated rats are depicted in Tables 3.20, 3.21 and 3.22, respectively.

3.8.1. Total -SH Groups:

The content of total -SH groups were reported to decrease significantly ($P<0.001$) in all the CNS regions following dimecron toxicosis (Table 3.20).

Spinal cord showed maximum decrement, of the order of -45%, as compared to cerebrum (-41%), cerebellum (-22%) and brain stem (-40%).

3.8.2. Non-protein Bound -SH Groups

Significant depletion ($P<0.001$) was observed in the concentration of NBP-SH groups in all the regions of the CNS, after exposure to dimecron, as shown in Table 3.21. A greater

TABLE 3.20

Perturbation in total sulfhydryl group contents in discrete CNS regions following dimecron intoxication (2.0 mg / Kg b. wt, ip x 7 days).

(Values, expressed as n moles/ g fresh tissue, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	11.60 ± 0.15	6.76 ± 0.36*	-41
Cerebellum	8.29 ± 0.24	6.46 ± 0.30*	-22
Brain stem	11.17 ± 0.09	6.68 ± 0.20*	-40
Spinal cord	10.85 ± 0.15	5.92 ± 0.27*	-45

Degree of significance: * $P < 0.001$

TABLE 3.21

Perturbation in free (Non-Protein Bound) sulfhydryl group contents in discrete CNS regions following dimecron intoxication (2.0 mg/Kg b. wt, ip x 7 days).

(Values, expressed as n moles/ g fresh tissue, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	4.16 ± 0.07	2.97 ± 0.13*	-27
Cerebellum	4.32 ± 0.04	2.80 ± 0.13*	-35
Brain stem	3.44 ± 0.08	1.88 ± 0.09*	-45
Spinal cord	3.39 ± 0.07	1.82 ± 0.11*	-46

Degree of significance: * $P < 0.001$

TABLE 3.22

Perturbation in Protein-Bound sulfhydryl group contents in discrete CNS regions following dimecron intoxication.

(Values, expressed as n moles/ g fresh tissue, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	7.48 ± 0.092	3.79 ± 0.405*	-49
Cerebellum	3.97 ± 0.193	3.66 ± 0.258^{NS}	-8
Brain stem	7.73 ± 0.060	4.80 ± 0.215*	-37
Spinal cord	7.46 ± 0.139	4.10 ± 0.345*	-45

Degree of Significance: * $P < 0.001$
NS = Not significant

depletion was noticed in brain stem (-45%) and spinal cord (-46%), as compared to cerebrum (-27%) and cerebellum (-35%).

3.8.3. Protein Bound -SH Groups:

Following dimecron intoxication, the content of protein-bound -SH groups were found to diminish significantly ($P<0.001$) in cerebrum, brain stem and spinal cord, cerebrum showing the maximum depletion (-49%). Cerebellum, however, showed a non-significant depletion (-8%).

3.9.0. Glutathione-S-transferase (GST) in Brain

3.9.1 Effect of Dimecron on Glutathione-S-transferase Activity:

Dimecron intoxication (2.0 mg / kg b. wt. ip x 7 days) led to a significant inhibition in the activity of GST ($P<0.001$) in all the CNS regions (Table 3.23).

Brain stem (-32%) and spinal cord (-42%) were reported to deplete more significantly, as compared to cerebrum (-19%) and cerebellum (-26%).

3.9.2. Protective Effect of Cithiolone Against GST Inhibition:

Fig. 3.7 shows the protective effect of cithiolone against the dimecron-induced inhibition of GST. When rats were treated with cithiolone (8.0 mg / kg b.w.t, ip) simultaneously with dimecron, no significant change was noted in the activity of GST in all the CNS regions and the

TABLE 3.23

Perturbation in the activity of glutathione-S-transferase in the CNS regions following dimecron toxicosis.

Data, expressed as n moles of CDNB conjugate/mg protein/min, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	173.92 ± 0.93	139.28 ± 0.75*	-19
Cerebellum	149.22 ± 1.88	109.81 ± 2.06*	-26
Brain stem	131.98 ± 1.78	88.93 ± 1.64*	-32
Spinal cord	160.60 ± 1.17	92.17 ± 0.94*	-42

Experimental: Dimecron (2.0 mg/ Kg b. wt, ip x 7 days)
Degree of significance: * $P < 0.001$

TABLE 3.24

Protective effect of cithiolone against the dimecron-induced alterations in glutathione-S-transferase activity in various CNS regions.

(Data, expressed as n moles of CDNB conjugate/mg protein/min, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	173.92 ± 0.93	170.79 ± 0.93	NS
Cerebellum	149.22 ± 1.88	146.80 ± 0.96	NS
Brain stem	131.98 ± 1.78	129.35 ± 0.88	NS
Spinal cord	160.60 ± 1.17	157.86 ± 0.86	NS

Experimental: Cithiolone (8.0 mg / Kg) + Dimecron (2.0 mg / Kg b. wt, ip x 7 days).
NS : Not Significant

TABLE 3.25

Alteration in the activity of glulathione-S-transferase in the CNS regions following exposure to cithiolone.

(Data, expressed as n moles of CDNB conjugate/ mg protein/ min, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	173.92 ± 0.93	199.64 ± 3.14*	14
Cerebellum	149.22 ± 1.88	173.73 ± 1.64*	16
Brain stem	131.98 ± 1.78	154.12 ± 1.38*	16
Spinal cord	160.60 ± 1.17	191.58 ± 0.44*	19

Experimental: Cithiolone (8.0 mg / Kg) + Dimecron (2.0 mg / Kg b. wt, ip x 7 days).
Degree of significance: * $P < 0.001$

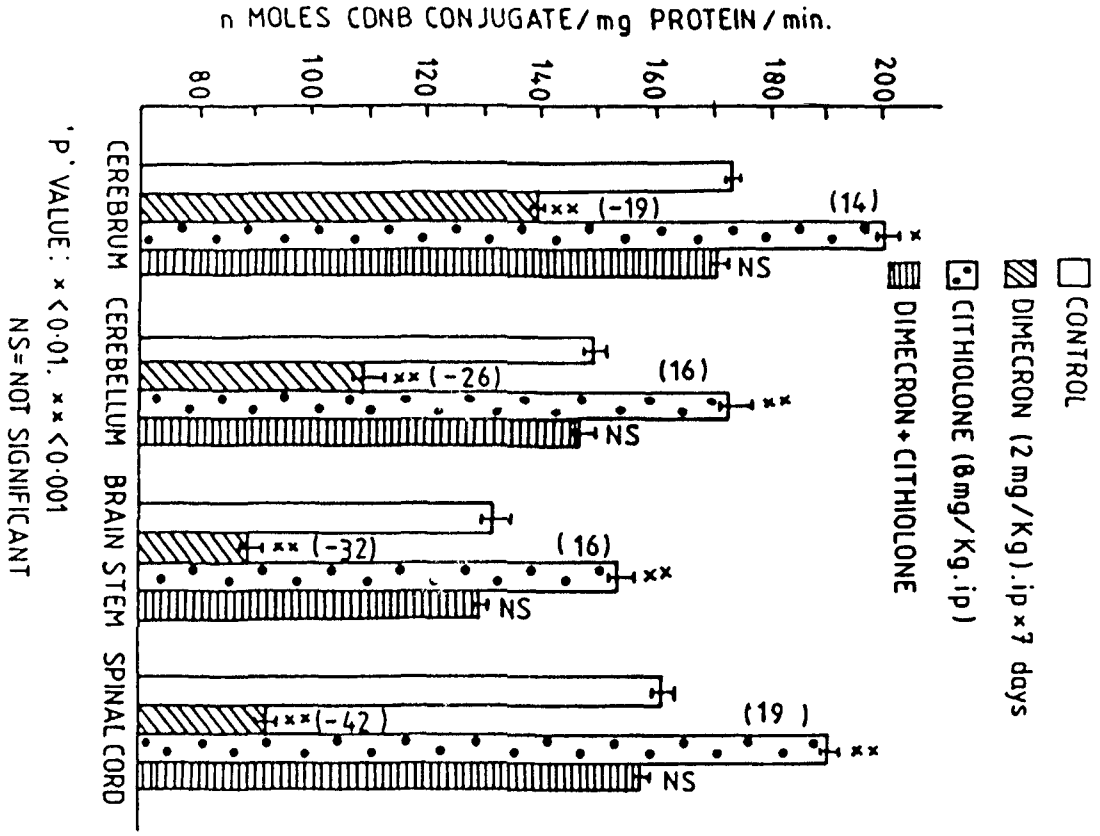


Fig.3·7. CITIOLONE INDUCED PROTECTION OF GST ACTIVITY AGAINST INHIBITION BY PHOSPHAMIDON INTOXICATION.

activity of the enzyme was seen to be near the control values (Table 3.24).

When treated with cithiolone alone (Table 3.25) however, the activity of GST was reported to enhance significantly ($P<0.001$) in cerebrum (14%), cerebellum (16%), brain stem (16%) and spinal cord (19%).

3.10.0 Superoxide Dismutase (SOD) in Brain

3.10.1 Effect of Dimecron on Superoxide Dismutase activity:

The activity of superoxide dismutase was found to decrease in all the CNS regions of dimecron-treated (2.0 mg/kg b. wt. ip x 7 days) rats (Table 3.26).

SOD activity was inhibited in cerebrum ($P<0.02$), cerebellum, brain stem and spinal cord ($P<0.001$). Inhibition was noticed to be more prominent in brain stem (-35%) and spinal cord (-48%), compared to cerebrum (-16%) and cerebellum (-22%).

3.10.2 Protective Effect of Cithiolone Against SOD Inhibition:

Protective effect of cithiolone against dimecron-induced inhibition of SOD is described in Fig. 3.8. Following the simultaneous treatment of cithiolone (8.0 mg / kg b.wt, ip) and dimecron, no significant change in the SOD activity was observed in any of the investigated CNS regions (Table 3.27).

However, cithiolone intoxication alone, was reported to induce significant increment of the SOD activity in cerebrum

TABLE 3.26

Perturbations in the activity of superoxide dismutase in various CNS regions following dimecron intoxication.

(Data, expressed as units/mg tissue/min, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	0.302 ± 0.014	0.253 ± 0.007*	-16
Cerebellum	0.485 ± 0.014	0.377 ± 0.007**	-22
Brain stem	0.676 ± 0.010	0.435 ± 0.013**	-35
Spinal cord	0.859 ± 0.012	0.454 ± 0.007**	-48

Experimental: Dimecron (2.0 mg / Kg b. wt, ip x 7 days).
Degree of significance: * $P < 0.02$, ** < 0.001

TABLE 3.27

Protective effect of cithiolone on dimecron-induced alteration in the activity of superoxide dismutase in the CNS regions.

(Data, expressed as units/ mg tissue/ min, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	0.302 ± 0.014	0.299 ± 0.007	NS
Cerebellum	0.485 ± 0.014	0.481 ± 0.009	NS
Brain stem	0.676 ± 0.010	0.658 ± 0.007	NS
Spinal cord	0.859 ± 0.012	0.862 ± 0.010	NS

Experimental: Cithiolone (8.0 mg/Kg) + Dimecron (2.0 mg/Kg b. wt, ip x 7 days).
NS: Not significant

TABLE 3.28

Effect of cithiolone (8.0 mg / Kg b. wt, ip x 7 days) on superoxide dismutase activity in the CNS regions.

(Data, expressed as units/ mg tissue, are mean ± SE of six animals).

CNS regions	Control	Experimental	% change
Cerebrum	0.302 ± 0.014	0.352 ± 0.001*	16
Cerebellum	0.485 ± 0.014	0.578 ± 0.003**	19
Brain stem	0.676 ± 0.010	0.815 ± 0.005**	20
Spinal cord	0.859 ± 0.012	1.062 ± 0.027**	23

Experimental: Cithiolone (8.0 mg / Kg b. wt, ip x 7 days).
Degree of significance: * $P < 0.01$, ** $P < 0.001$

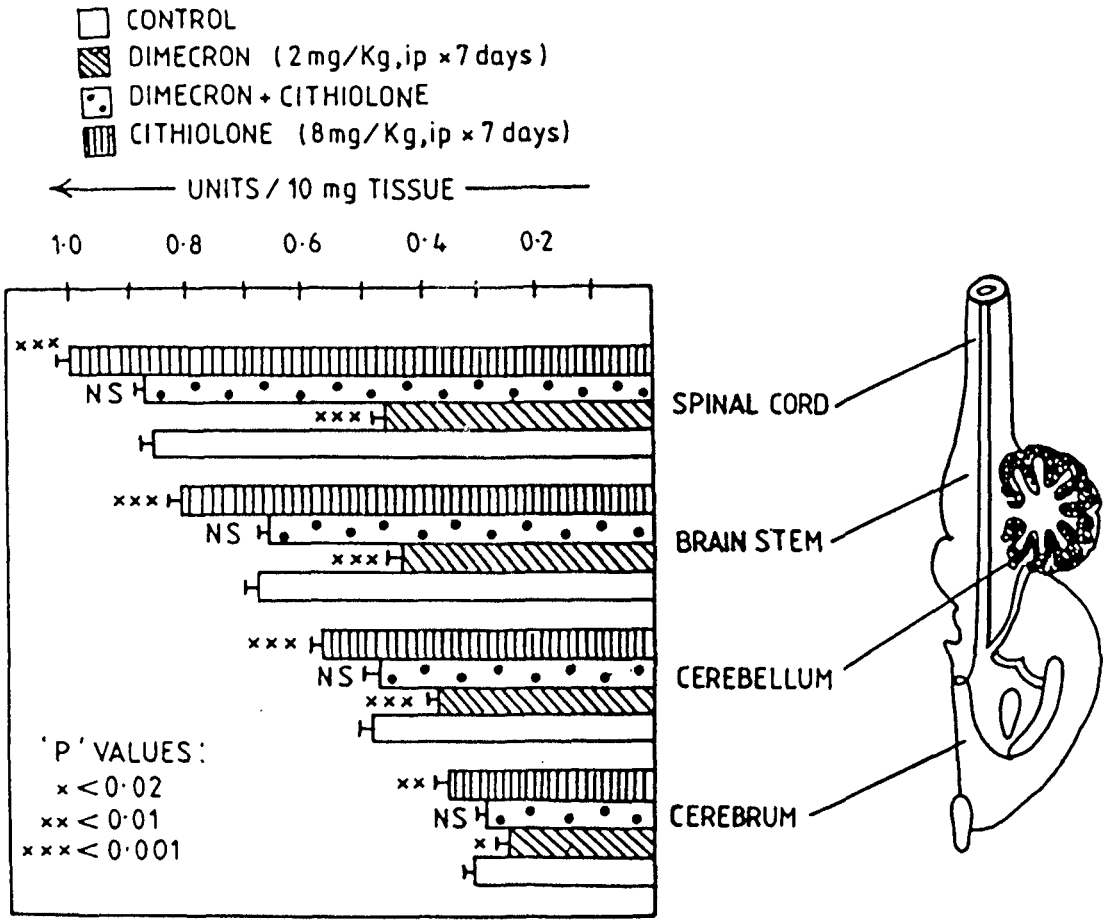


Fig.3·8. EFFECT OF DIMECRON ON SOD ACTIVITY IN THE CNS: PROTECTION BY CITHIOLONE.

(P<0.01), cerebellum, brain stem and spinal cord (P<0.001), with maximum elevation found in the spinal cord (+23%), as shown in Table 3.28.

3.11.0. Nucleic Acids in Brain

3.11.1 Effect of Dimecron on DNA

The concentration of deoxyribonucleic acid (DNA) was found depleted significantly (P<0.001) in cerebrum, cerebellum, brain stem and spinal cord of dimecron-treated (2.0 mg/kg b. wt. ip x 7 days) rats (Table 3.29). Cerebrum showed the greatest decrement in the DNA content (-45%).

3.11.2 Effect of Dimecron on RNA:

Table 3.30 reveals neurochemical alteration in the levels of ribonucleic acid (RNA) following dimecron toxicosis (2.0 mg / kg b. wt. ip x 7 days). RNA content was reported to increase in cerebrum (P<0.01), cerebellum, brain stem and spinal cord (P<0.001). Maximum increment (33%) was seen in the spinal cord.

3.12.0. Effect of Dimecron on Brain Protein Level

Dimecron intoxication (2.0 mg/kg b. wt.ip x 7 days) led to significant decrease (P<0.001) in the total protein contents of cerebrum, cerebellum, brain stem and spinal cord, as depicted in Table 3.31. Protein depleted most prominently in the cerebrum (-22%).

TABLE 3.29

Alteration of DNA levels in different regions of the rat CNS following the administration of dimecron (2.0 mg/ Kg b. wt, ip.) daily for 7 days.

Values, expressed as mg/g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	1.89 ± 0.07	1.03 ± 0.01*	-45
Cerebellum	5.24 ± 0.33	4.13 ± 0.03*	-21
Brain stem	1.82 ± 0.04	1.34 ± 0.02*	-26
Spinal cord	1.56 ± 0.09	1.12 ± 0.01*	-28

Degree of significance: * $P < 0.001$

TABLE 3.30

Alteration of RNA levels in different regions of the rat CNS following the administration of dimecron (2.0 mg/ Kg b. wt, ip.) daily for 7 days.

Values, expressed as mg/g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	3.78 ± 0.08	4.68 ± 0.05*	+23
Cerebellum	4.30 ± 0.06	5.25 ± 0.03**	+22
Brain stem	3.69 ± 0.06	4.73 ± 0.03**	+28
Spinal cord	3.05 ± 0.02	4.08 ± 0.02**	+33

Degree of significance: P < * 0.01, ** 0.001

TABLE 3.31

Alteration of protein levels in different regions of the rat CNS following the administration of dimecron (2.0 mg/ Kg b. wt, ip.) daily for 7 days.

Values, expressed as mg/g fresh tissue, are mean ± SE of six animals)

CNS regions	Control	Experimental	% change
Cerebrum	152.15 ± 1.63	118.39 ± 1.87*	-22
Cerebellum	223.82 ± 3.05	183.38 ± 0.56*	-18
Brain stem	220.40 ± 2.68	185.86 ± 2.06*	-15
Spinal cord	139.96 ± 3.61	117.55 ± 1.05*	-16

Degree of significance: * $P < 0.001$

3.13.0 Accumulation of Dimecron in the CNS:

Dimecron was recovered, using thin layer chromatography (TLC), from various CNS regions of the rats intoxicated with three graded doses of dimecron (1.0, 1.5 and 2.0 mg / kg b. wt, ip x 7 days). The Rf value of dimecron, as evaluated from the spots of dimecron which appeared on the TLC plates (Fig. 3.9), was found to be equal to 0.36.

A remarkable dose-related elevation was observed in the concentration of dimecron accumulated in all the CNS regions, i.e., cerebrum, cerebellum, brain stem and spinal cord (Table 3.32).

Cerebrum showed maximum percent recovery compared to concentration of dose injected, in all the three dose groups (22.2, 22.3 and 24.2%, respectively).

Trends of ANOVA revealed that all the variations, between various regions as well as between different doses, were highly significant ($P<0.001$).

Fig 3.9a Photograph of thin-layer chromatogram showing separated spots of dimecron (phosphamidon) and its metabolite desethylphosphamidon, detected by TLC from discrete CNS regions.
P: Phosphamidon spot, **D:** Desethylphosphamidon spot

CB,CL, BS and **SC:** Origin points of samples from cerebrum, cerebellum, brain stem and spinal cord, respectively.

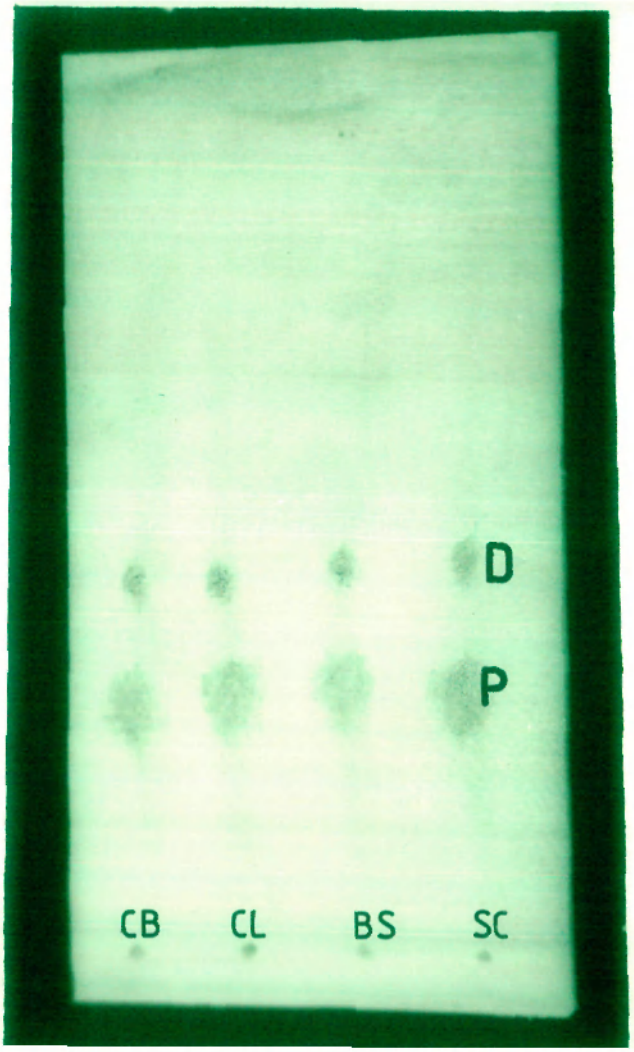

D
P
CB
CL
BS
SC

TABLE 3.32

Dose-related accumulation of dimecron in the CNS regions following its exposure for 7 days.

(Values, expressed as ug/g fresh tissue, are mean ± SE of 6 animals)

CNS regions	Dose (mg/Kg b. wt, ip)		
	1.0	1.5	2.0
Cerebrum	0.222 ± 0.006 (22.2)	0.334 ± 0.007 (22.3)	0.483 ± 0.006 (24.2)
Cerebellum	0.152 ± 0.004 (15.2)	0.243 ± 0.002 (16.2)	0.356 ± 0.002 (17.8)
Brain stem	0.177 ± 0.002 (11.7)	0.171 ± 0.008 (11.4)	0.240 ± 0.008 (12.0)
Spinal cord	0.123 ± 0.003 (12.3)	0.201 ± 0.007 (13.4)	0.281 ± 0.002 (14.0)

Figures in parentheses indicate percent uptake compared to dose concentration.
*Analysis of Variance (ANOVA): Between regions (rows): $P < 0.001$; Between doses (columns): $P < 0.001$

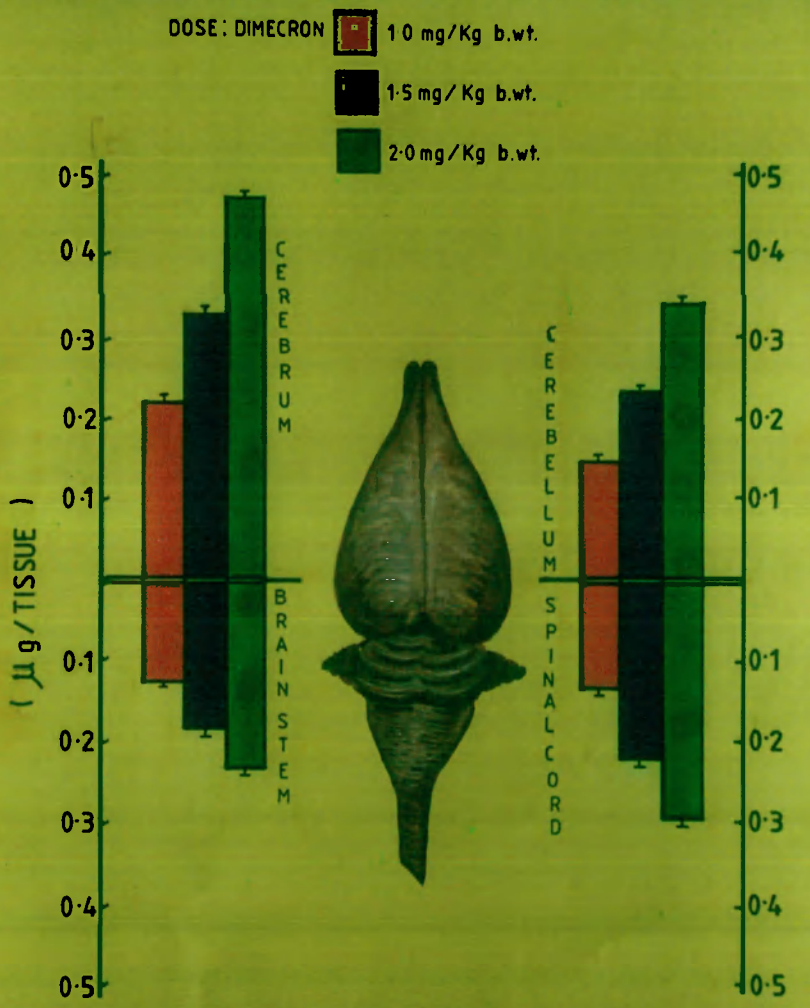


Fig.3·9. DOSE-RELATED ACCUMULATION OF DIMECRON IN THE CNS. AS DETECTED BY T.L.C. .

4. DISCUSSION

Organophosphate pesticides have been extensively studied regarding their neurochemical and neurobehavioral effects on the non-target organisms. To date, very little literature is available, however, on the neurotoxicity of dimecron (phosphamidon), a widely used, potent but relatively less studied organophosphate pesticide.

The present study demonstrates regional heterogeneity not only related to the effects of dimecron on various neurochemical and neurobehavioral parameters, but also in the extent of these adverse effects on the different regions of the CNS. These regional variations might be explained keeping in view the fact that the brain is a very complex organ composed of many structural and functional components with markedly different and independently regulated levels of functional and metabolic activities. It is difficult to assess the levels of functional and metabolic activities at the same time (Sokoloff 1979). This heterogeneity of the brain is of great importance in the evaluation and interpretation of the biochemical findings (Hertz 1969).

4.1.0 Acetylcholinesterase:

Acetylcholinesterase (AChE) is regarded as a tool for quantification of organophosphate induced effects (Voss 1968). Although, quite a large number of previous studies have reported inhibition of AChE by OP poisoning, very little

information is available regarding neurotoxic effect of dimecron on the AChE activity.

The present study discloses a remarkable depletion of AChE activity in various CNS regions following dimecron toxicosis (Table 3.4).

Most of the organophosphorus compounds act by disrupting the nerve impulse due to the accumulation of acetylcholine (ACh) at the synapses following AChE inhibition (Paul et al 1979). This supports the inhibition of AChE reported in this study. Our results are also supported by a number of previous reports (DuBois et al 1979; Emsley et al 1976; Paul et al 1979; Hall and Kolbe 1980) revealing AChE depletion after organophosphate poisoning. From this Laboratory, Ali et al (1977) have also reported inhibitory effect of dichlorvos on the AChE activity. Emsley et al (1976) suggested that the OP pesticides inhibit the activity of AChE through crossing-over the blood-brain barrier (BBB), which is also supported by a recent finding of Naqvi and Hasan (1990), who showed dose-dependent accumulation of dimecron in the central nervous system, obviously by crossing the BBB.

Accumulation of ACh at the synapses, resulting from AChE inhibition, has been suggested to induce cholinergic excitation which causes reduction of the monoaminergic

neurotransmitters (Tracy et al 1960). Dimecron has been found to deplete the levels of brain monoamines (Naqvi and Hasan 1991b), which also endorses the AchE reduction (Naqvi and Hasan 1991c). Brezezinski (1978) and Brezezinski and Paruszewska (1980) have also reported decrease in the brain neurotransmitter levels in the organophosphate-treated rats. Moreover OP-induced increase in the content of acetylcholine was reported by Hall and Kolbe (1980), which is also consistent with the depletion of acetylcholinesterase.

Previously, it was assumed that the AChE inhibition is an irreversible process (Weiss 1959; Murphy et al 1968; O'Brien 1976). Subsequent studies, however pointed out the inhibition of AChE following organophosphate toxicity, may be reversed also (Muthazhagu 1985; Khan 1989).

Interestingly, the present study reveals a remarkable protection against dimecron-induced depletion of AChE in all the CNS regions when the rats were treated with cithiolone simultaneously with dimecron (Table 3.5). This can be explained by the fact that cithiolone (acetyl homocysteine thiolactone), an antioxidant, probably dissociates the AChE-anti AChE complex, leading to reactivation or detoxification of acetylcholinesterase. Dissociation of ChE-anti ChE complex has been found to play a very vital role in the detoxification process (Lauwerys and Buchet 1971). The stability of the phosphorylated enzyme depends upon the

nature of the pesticide (Reiner 1971). Moreover, cithiolone (acetylhomocysteine thiolactone) has been reported to protect against dimecron neurotoxicity (Naqvi and Hasan 1991a). Elevation in the activity of AChE in the CNS regions of the cithiolone-intoxicated rats (Table 3.6) also supports the suggestion that cithiolone reactivates the enzyme activity.

4.2.0 Monoamine Metabolism and Neurobehaviour:

4.2.1 Monoamines and Monoamine Oxidase:

Monoamines, consisting of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) act as important neurotransmitters in the neuronal transmission system.

Dimecron intoxication has been found to significantly diminish the levels of DA, NE and 5-HT in the cerebrum, cerebellum, brain stem and spinal cord, as reported in this study (Tables 3.7, 3.8 and 3.9).

It has been universally accepted that the organophosphate-induced inhibition of AChE results in accumulation of acetylcholine (ACh) at the synapses (Paul et al 1979), leading to disruption of nerve impulses. This accumulation of ACh has been reported to stimulate cholinergic excitation, which in turn, may deplete the monoamine levels (Tracy et al 1960; Burn and Rand 1965; Brezezinski 1972). This is well in agreement with our results (Tables 3.7, 3.8, 3.9) revealing significant decrement in the concentration of DA, NE and 5-HT in cerebrum, cerebellum,

brain stem and spinal cord following dimecron intoxication (Naqvi and Hasan 1991b). Decreased monoamine levels in the CNS, following toxicosis with organophosphate dichlorvos, has also been reported by Ali et al (1980). They have, additionally, correlated depletion of 5-HT with increased motor activity, as 5-HT is said to account for enhanced motor activity (Scheckel et al 1969).

Monoamine oxidase (MAO) plays an important role in the metabolism of biogenic amines and in the behaviour (Shellenberger and Walaszec 1972; Chase and Murphy 1973). It is involved in the enzymatic deamination and subsequent degradation of biogenic amines (Cooper et al 1978). Elevation of MAO activity in various regions of dimecron-poisoned rats (Table 3.10) can, therefore, be correlated with the diminution of brain monoamine levels reported in this study (Naqvi and Hasan 1991 b). Alteration of MAO activity has been associated with convulsive seizures (Kilien and Frey 1973), which is supported by severe convulsions following dimecron poisoning, reported in this study. Moreover, Beeman and Matsumura (1973) have also shown that MAO activity is inversely proportional to the monoamine levels. Interestingly, maximum increment of MAO activity was observed in cerebellum which is in concordance with maximum depletion of DA content in that region. Concomitantly, the brain-stem showed minimum MAO enhancement and DA decrement. This can be

explained on the basis of the suggestion that the gray matter-rich cerebellum is likely to be affected by a toxicant more severely compared to white matter-rich brain stem (Naqvi and Hasan 1990), because the blood flow in the gray matter is reported to be many times higher than in the white matter (Landau et al 1955). However, in the cerebrum, which is also a gray matter-rich region, comparatively lower diminution of monoamines was observed. This contradiction may be explained by the higher lipid content of cerebrum as compared to cerebellum (Naqvi et al 1988; Gupta and Hasan 1988). This suggests a greater white matter fraction in the cerebrum, and hence the lesser diminution of monoamines in that region is not surprising, owing to known differences in the blood perfusion rates of cerebellum and cerebrum.

Interestingly, a remarkable cithiolone-induced protection against the MAO elevation was noticed in all the CNS regions (Fig. 3.5). The activity of MAO was reported to be near the control values in the CNS regions of rats subjected to simultaneous treatment of cithiolone and dimecron (Table 3.11). This protection may be the result of detoxication against dimecron neurotoxicity by cithiolone, a potent antioxidant which has been reported to protect against adverse effects of dimecron on the CNS (Hasan et al 1987; Naqvi and Hasan 1991a). Moreover when the rats were exposed to cithiolone only, MAO activity inhibited in all the regions (Table 3.12). It is, therefore, also possible that the

reported protection might be due to ability of cithilone to deplete the MAO activity and thus neutralizing the dimecron-induced elevation of the enzyme.

4.2.2. Open Field Behaviour:

According to Parale and Kulkarni (1986), the organophosphate-induced cholinergic stimulation in the brain leads to reduction of the central adrenergic activity, which is very important in the pathogenesis of behaviour. Furthermore, it has been suggested that alterations in the biochemical mechanisms and biogenic amine concentrations manifest varying disorders in the behaviour (Taylor and Snyder 1971; Kaloyanova et al 1979).

Accordingly, the present study reports a remarkable change in the open field behaviour (OFB) of the dimecron-treated rats. Significant day-to-day decrement was observed in the scores of OFB parameters, i.e. ambulation, rearing and preening (Naqvi & Hasan 1991b).

Ambulation and rearing are known to be horizontal (simple) and vertical (complex) stereotypy, respectively (Dandiya et al 1969; Gupta and Holland 1972). Decrement of ambulation (Table 3.1) and rearing (Table 3.2) scores can therefore be correlated with the depleted DA content (Table 3.7), as DA in the main modulator of stereotype behaviour (Kulkarni and Dandiya 1972). Rearing is also indicative of

cortical stimulation (Lat 1965), while preening response has been described as a behavioural correlate of cortical stimulation (Gupta et al 1971). Depression of preening score (Table 3.3) can therefore be related to the diminution of cortical stimulation, i.e. rearing. Our results (Naqvi & Hasan 1991b) are further supported by the findings of Ali et al (1980), showing decrease in the OFB parameters alongwith depletion of brain monoamines following intoxication with organophosphate, dichlorvos. The dose-response signs of dimecron toxicity, viz. fasciculations, convulsions, ataxia and lethargy, are also in agreement with decrease in the ambulation, rearing and preening scores.

Remarkable protection against the behavioral changes was observed following simultaneous treatment with cithiolone alongwith dimecron. Scores of ambulation (Fig. 3.1), rearing (Fig. 3.2) and preening (Fig. 3.3) showed no significant alteration throughout the seven days of exposure. This protection is well supported by the detoxifying ability of antioxidant cithiolone against dimecron neurotoxicity (Hasan et al 1987; Naqvi and Hasan 1991a). Moreover, protection against dimecron-induced elevation of MAO (Fig. 3.5) are also in concordance with the protection against behavioral changes.

4.3.0 Brain Lipids:

Findings of the present investigation reveal decrement in the levels of total lipids, phospholipids, cholesterol and

gangliosides following the dimecron toxicosis (Naqvi et al 1988).

4.3.1. Total Lipids:

Brain is one of the richest in lipids among the various body organs. Lipids account for about half the dry weight of most of the structural architecture of membranes in the brain (Ordy and Kaack 1975). They also constitute components of the ion channels, portions of the neurotransmitter receptors, and are major constituents of myelin. Myelin sheath and neuropil of gray matter account for much of the total lipid contents of the brain tissue (Scharf 1953; Schimizu 1965), making upto 65% of the weight of white matter and 35-40% of gray matter (Brante 1949). Lipids of various tissues are known to be in a dynamic steady state and there is continuous replacement of existing molecules by new ones (White et al 1959). It is noteworthy that distinct regional differences occur in lipid contents and its turn-over in various pathways and centres of the mammalian brain. Alterations in the lipid metabolism may be due to changes in the rate of anabolism, catabolism, or both. These processes are regulated by the activities of appropriate enzymes.

The dimecron-induced depletion of total lipid content (Table 3.13) are supported by the findings of Majno and Karnovsky (1955) who reported perturbed lipid synthesis in the brain after organophosphate poisoning. Diminution of

total lipids suggests a possible increase in the degradation of lipids, as evident by the increased rate of lipid peroxidation and lipase activity following organophosphate toxicity (Islam et al 1983; Tayyaba and Hasan 1985; Hasan and Khan 1985; Vadhwa and Hasan 1986; Naqvi et al 1988). Ham and Rose (1969) and Hazzard et al (1969) have suggested that changes in the lipase activity may be an important factor contributing to perturbed plasma lipid levels. Moreover, Haider et al (1981) have also correlated decreased lipid contents in various regions of the brain with enhanced lipase activity in those regions.

4.3.2. Phospholipids:

Phospholipids play an important role in the CNS as essential membrane constituents. The properties of a membrane, therefore depend, to some extent, on the phospholipid composition. The myelin of phospholipids remain almost stable throughout the life span of the animal (Davison and Dobbing 1960). Individual lipid classes, specially membrane phospholipids are undergoing constant turnover at different rates with respect to the structure of the lipid and localization in various cells and membranes (Porcellati 1983). The depletion of phospholipid levels in different CNS regions of dimecron-treated rats (Table 3.14), can be supported by the findings of Nelson and Barnum (1960) who observed inhibition of phospholipid biosynthesis by the

administration of diisopropylfluorophosphate (DFP). Moreover, Tayyaba and Hasan (1980) have also reported decrement of phospholipid contents in the CNS regions following metasystox intoxication, which also endores present findings.

4.3.3. Cholesterol:

Cholesterol is present in large amounts in the CNS. It occurs in white matter in amounts exceeding those in gray matter (Brante 1949). It is the characteristic lipid of myelin sheath. The constant amount of cholesterol in the brain suggests that the sterol is metabolically stable and is thus excluded from the normal dynamic exchange process common to almost all other body constituents (Waelsch et al 1940). About 70% of the total brain cholesterol is present in the myelin (Dobbing 1964). Demyelination caused by organophosphate toxicity or multiple sclerosis leads to loss of cholesterol esters (Davison and Wajda 1962). Moreover, Majno and Karnovsky (1961) observed that mipafox, an OP pesticide, inhibited the incorporation of acetate, choline and phosphate into the lipids of CNS. This is well in agreement with the dimecron-induced decrease in the content of cholesterol in various regions of the CNS (Table 3.15).

4.3.4 Gangliosides:

Gangliosides are known to be involved in the synaptic transmission, since they act as receptor sites for various neurotoxins (North et al 1961). Gangliosides are generally

synthesised in neuronal perikarya and are transported to the nerve endings along with macromolecules (Rahman and Rosner 1973; Ledeen et al 1976; Landa et al 1979), but local synthesis within the axons and nerve endings (Ledeen et al 1976) or even at the plasma membrane level (Preti et al 1980) can not be excluded. Gangliosides are essential membrane constituents and are more concentrated and complex in the CNS than in any other organ (Rahman 1983). Gangliosides may bind biologically active compounds as well as various neurotoxins, through their terminal N-acetylneuraminic acid moiety (Avrova 1971).

To our knowledge, no previous report is available on the neurochemical effect of dimecron on brain gangliosides. However, the degradation of gangliosides in various CNS regions following dimecron intoxication (Table 3.16) can be explained by the suggestion of Ledeen and Mellanby (1977), that the reduction of gangliosides occurs through sequential removal of neuraminic acid by neuraminidases. Findings of Islam et al (1983), revealing metasystox-induced decrement of brain ganglioside contents, also support the results of this study (Table 3.16). Depletion of gangliosides has also been reported by methyl parathion toxicosis (Khan and Hasan 1988).

Degradation of brain gangliosides (Naqvi et al 1988) can also be correlated with the dimecron-induced perturbations in the open filed behaviour (Naqvi and Hasan 1991b), as Irwin and Samson (1971) have suggested that

certain types of behavioral stimulations seem to be accompanied by alterations in the ganglioside metabolism.

4.4.0. Lipid Peroxidation:

Investigation of lipid peroxidation is a recently applied approach to the study of neurotoxicity (WHO 1986). Lipid peroxidation is the basic mechanism of oxidative destruction of polyunsaturated fatty acid chains in biological membranes. It involves direct reaction of oxygen and lipid molecule which leads to the formation of free radical intermediates, and subsequently semi-stable peroxides.

Biomembranes and subcellular organelles are the major sites of lipid peroxidation damage (Tappel 1970). Among the different tissues, brain has been reported to show considerably high degree of peroxidation, probably due to the fact that brain has the highest content of lipids compared to other organs (Kartha and Krishnamurthy 1978). Lipid peroxidation appears to be a phenomenon that reflects free-radical events associated with biological membranes which contain most of the polyunsaturated fatty acid-containing lipids. The brain homogenate has apparently the necessary unsaturated fatty acids and the catalysts for peroxidation in the architecture of the cell itself, which are readily available for reaction with molecular oxygen to undergo lipid peroxidation.

Free radical mechanism leading to lipid peroxidation and degradation of brain lipids with the loss of membrane integrity is considered to be important factor in the development of irreversible damage of brain cells during ischaemic and other adverse conditions (Demopoulos et al 1979).

Following intoxication with dimecron, lipid peroxidation was found enhanced in all the regions of the CNS (Table 3.17). This enhancement (Naqvi et al 1982) can be supported by the findings of Hasan and Ali (1981) disclosing increment of the rate of lipid peroxidation following administration of organophosphate dichlorvos. Similar results have been observed by intoxication with metasystox (Islam et al 1983; Tayyaba and Hasan 1985) and methyl parathion (Hasan and Khan 1985). Furthermore, Haider et al (1982) have suggested that increment of lipid peroxidation might contribute to the decrement of lipid levels in various brain areas. Elevation of lipid peroxidation can also be correlated with depletion of the sulfhydryl group contents in the CNS regions following dimecron toxicity (Naqvi and Hasan 1991a), because it has been suggested that decrement of sulfhydryl groups may lead to deficient degradation of lipid peroxides to hydroxyacids, causing increment of lipid peroxides in the tissue (Tappel 1970).

Interestingly, an antioxidant agent, cithiolone, has been found to protect against dimecron-induced elevation of

lipid peroxidation (Fig. 3.6). When the rats were simultaneously treated with cithiolone and dimecron, no significant change was observed in the rate of lipid peroxidation in any of the CNS regions (Table 3.18). Lipid peroxidation was decreased, however, following the administration of cithiolone, alone (Table 3.19). Since lipid peroxidation is an oxidative process, its decrement by the antioxidant cithiolone can well be understood. It can be assumed that cithiolone may probably act as 'in vivo' lipid antioxidant, protecting unsaturated fatty acids in the tissue lipids against peroxidation. Bieri and Anderson (1960) suggested that each tissue has an "antioxigenic" potential which is determined by a balance between factors promoting peroxidation and by those exerting an antioxidant action. Protective effect of cithiolone against the OP induced increment of lipid peroxidation have also been reported by Hasan et al (1987) which support the present findings (Table 3.18). Moreover, Tayyaba and Hasan (1985) have observed protection against metasystox-induced elevation of lipid peroxidation by the simultaneous treatment of another antioxidant, Vitamin E.

It can, therefore, be concluded that the peroxidation of endogenous lipids was enhanced by dimecron intoxication, resulting in the fragmentation of lipids, thereby leading to disintegration of membrane structure and finally to cell

damage. This can however be protected by antioxidant, cithiolone, owing to its free-radical scavenging and antioxidative effects.

4.5.0. Sulfhydryl Groups:

Sulfhydryl (-SH) groups act as important enzymatic sites (Hoch and Vallee 1959). According to Chio and Tappel (1969), sulfhydryl enzymes are most susceptible to lipid peroxide-induced inactivation. Glutathione (GSH) has been reported to play a protective role against free radical-mediated or peroxidative damage (Stokinger and Coffin 1968; Little and O'Brien 1968; DeLucia et al 1972; Chow and Tappel 1972). GSH, which is a potent nucleophile, is involved in conjugation reactions which are important for detoxication against electrophilic toxicants (Chasseaud 1980). This conjugation reaction between -SH group of glutathione and the toxic compound, has been reported to be catalyzed by an enzyme glutathione-S-transferase (GST) (Grover and Sims 1964; Boyland and Chasseaud 1969; Habig et al 1974; Al-Turk et al 1987).

The present study reveals significant depletion in the concentration of total (Table 3.20), non-protein bound (Table 3.21) and protein bound (Table (3.22) sulfhydryl groups in all the CNS regions following dimecron intoxication. This depletion may be supported by our findings indicating

dimecron-induced decrement in the activity of GST in the CNS regions (Naqvi and Hasan 1991a).

Moreover, since sulfhydryl groups are involved in the cellular detoxication against peroxidative damage (Chow and Tappel 1972), the diminution of sulfhydryl group contents can also be correlated with dimecron-induced enhancement of lipid peroxidation in the CNS (Naqvi et al 1988). This can be explained by the suggestion of Tappel (1970) that depletion of sulfhydryl groups may lead to deficient degradation of lipid peroxides to hydroxy acids, resulting in the increment of lipid peroxide contents.

4.6.0 Glutathione-S-transferase:

Glutathione-S-transferase (GST) represents an important class of xenobiotic metabolising enzymes (Mukhtar et al 1981). GST has been shown to be present in the mammalian and avian brain, besides its wide distribution in the body (Dixit et al 1981). Brain GST has been reported to play an important role in the detoxication of potential toxicants through their conjugation and biotransformation (Booth et al 1961; Boyland and Chasseaud 1969; Wood 1970; Dixit et al 1980). Jakoby and Keen (1977) have proposed that GST provides a "sacrificial" covalent linkage for electrophilic neurotoxic compounds.

Our findings indicating dimecron-induced inhibition of GST in all the CNS regions (Table 3.23), can, therefore, be

the result of cumulative effect of dimecron, inhibiting its GST-mediated biotransformation pathway. This may be a critical factor for the neurotoxic effect of any toxicant, which requires disposal through GST conjugation. Moreover, Boyland and Chasseaud (1969) and Das et al (1982) have suggested that greater accumulation of the toxic compound may inhibit the GST. Dimecron readily crosses the blood-brain barrier and we have already reported its accumulation in the CNS following graded dosing (Naqvi and Hasan 1990), which clearly supports GST inhibition following dimecron toxicosis.

GST has been reported to initiate detoxication process by catalyzing the conjugation reaction between the electrophilic toxicant and sulfhydryl group of glutathione (GSH), a nucleophile (Grover and Sims 1964; Boyland and Chasseaud 1969; Habig et al 1974). The dimecron-induced depletion of the GST activity may also be correlated with diminution of the level of sulfhydryl groups in the CNS regions following administration of dimecron (Naqvi and Hasan 1991a).

Cithiolone, an antioxidant compound, however was reported to protect against the dimecron-induced inhibition of GST (Fig. 3.7). The activity of GST was observed to be near the control values in all the CNS regions of the rats subjected to simultaneous treatment of cithiolone and dimecron (Table 3.24). Notably, the GST activity was enhanced

when cithiolone was administered alone (Table 3.25). This suggests that the protection of GST may probably be due to reactivation of the enzyme by cithiolone, thereby enhancing its capability to initiate detoxication against dimecron toxicity (Naqvi and Hasan 1991a).

It can be concluded that the inhibition of brain GST, resulting from binding of the enzyme by dimecron, may lead to an overall suppression of the biotransformation and detoxication reaction, resulting in an increased vulnerability of the brain regions to the exposure of dimecron toxicosis. Antioxidant, cithiolone interestingly protects against dimecron neurotoxicity by reactivating and hence increasing the detoxication potential of GST.

4.7.0. Superoxide Dismutase:

The activity of superoxide dismutase (SOD) was found to diminish in cerebrum, cerebellum, brain stem and spinal cord of the dimecron-treated rats (Table 3.26).

Superoxide dismutase activity is universally present in respiring cells. The substrate is an unstable free radical that can be present only in minuscule amounts at any instant. It catalytically scavenges the superoxide radicals (Fridovich 1972). The SOD activity is said to be a natural defence mechanism against oxidative damage to the tissue (Fridovich 1975).

Todate, no report is available on the effect of dimecron on the activity of SOD. However, dimecron has been reported to induce elevation of peroxidative degradation of brain lipids, i.e. lipid peroxidation (Naqvi et al 1988). The dimecron-induced inhibition of SOD activity can, therefore be related to the enhanced lipid peroxidation in various CNS regions following dimecron toxicosis (Table 3.17), because SOD is known to protect against peroxidative damage. Dimecron has also been reported to enter the blood-brain barrier (Naqvi and Hasan 1990) and deplete -SH groups, another protective agents against peroxidative damage (Naqvi and Hasan 1991a). This is also in concordance with the diminution of SOD, reported here.

Acetylhomocysteine thiolactone (cithiolone), however, revealed remarkable protective effect against inhibition of SOD (Fig. 3.8). Combined administration of cithiolone and dimecron showed significant protection of SOD activity (Table 3.27), while the activity of SOD enhanced by cithiolone intoxication (Table 3.28). This protection might be the result of reactivation of SOD by cithiolone, through its antioxidative action, and hence enabling SOD to protect against dimecron-induced peroxidative damage. Interestingly, cithiolone has already be found to protect against dimecron-induced enhanced lipid peroxidation in the CNS (Hasan et al 1987), which also supports our findings.

4.8.0 Nucleic Acids

The dimecron-intoxication was reported to induce significant perturbations in the levels of nucleic acids viz. deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) in various regions of the CNS.

4.8.1 Deoxyribonucleic Acid (DNA):

Significant diminution was observed in the concentration of DNA in cerebrum, cerebellum, brain stem and spinal cord after toxicosis with dimecron (Table 3.29). One of the explanations for this depletion may be the degeneration of neurons and nerve fibres by the organophosphate toxicity, as suggested by Hasan et al (1979). Our results agree with a number of previous studies regarding the effects of other organophosphate pesticides on the DNA contents of the brain. Phosalone has been reported to decrease DNA levels in various brain regions (Kirubagaran 1984; Palanivelu 1984; Tamilvanan 1984). Decrement of DNA levels in various CNS regions following dichlorvos intoxication has also been reported (Paolo and Fini 1960). Moreover, Dean (1972) observed diminution of the DNA concentration after dichlorvos toxicosis on cultured human lymphocytes.

Different degrees of dimecron-induced depletion was noticed in different CNS regions. This shows different vulnerability of each region towards dimecron toxicosis.

Cerebellum showed highest DNA concentration as well as maximum dimecron-induced depletion, which is well in accordance with the findings of May and Grenell (1959) which indicate that cerebellum contains highest DNA content. This high level of DNA in cerebellum reflects the extreme cell density of cerebellar granular layer.

Furthermore, the decrement of DNA contents reported in the present study can also be correlated with the findings of Tayyaba et al (1981) revealing metasystox-induced diminution of DNA alongwith elevation of the activity of DNase, the principal enzyme involved in the degradation of DNA.

The depletion of DNA contents in various CNS regions therefore, might be due to dimecron-induced perturbation in the metabolism of DNA, in addition to the degenerative changes.

4.8.2. Ribonucleic Acid (RNA):

In contrast, however, the response of RNA towards dimecron intoxication was entirely different from that of DNA. The RNA levels showed a significant increment in all the CNS regions following administration of dimecron (Table 3.30).

This increase of RNA concentration may be explained on the basis of the suggestion of Heath (1961), that

3.31). This can be explained on the basis of the suggestion of Hyden and Lange (1972) that increased neuronal activity decreases or inhibits the protein synthesis. Also, Ahmad et al (1978) have observed higher utilization of protein due to increased proteolytic activity, which results in decrement of protein contents.

Moreover, Bergen et al (1974) have correlated elevated RNA levels with increased nutrition or utilization of protein, which also supports the dimecron-induced depletion of protein (Table 3.31). It has been observed that many environmental and nutritional factors may perturb the brain protein (McIlwain and Bachelard 1971). Arnaiz et al (1975) are of the view that inhibition of protein level might be an indication of a disequilibrium in the normal energy-yielding metabolism. Free amino acids in the brain are known to be involved in a number of metabolic processes. Hence the increased demand for amino acids possibly may induce the break-down of proteins, as suggested by Neame (1968).

The dimecron-induced reduction in the protein levels in all the CNS regions of rats, is in accordance with similar findings with other OP pesticides. Tayyaba et al (1981) observed depletion of proteins in the brain and spinal cord of the rats intoxicated with metasystox. Phosalone has been reported to diminish the protein levels in the CNS regions of rats (Kirubagaran 1984; Palanivelu 1984; Tamilvanan 1984) and

demyelination, an important feature of organophosphate poisoning, results in the increased production of RNA. Organophosphates have also been found to induce degenerative changes in neurons and nerve fibres (Hasan et al 1979). Interestingly, it has been demonstrated that the synthesis of RNA increases due to damage of neurons (Mcllwain and Bachelard 1971).

Our results are also in agreement with previous studies revealing increased RNA concentration after toxicosis with other organophosphate pesticides (Kirubagaran 1984; Palanivelu 1984; Tamilvanan 1984).

Bergen et al (1974) suggested that elevation of RNA levels may be associated with improvement of protein nutrition or tissue function which leads to decrement of protein. Interestingly, dimecron has also found to deplete the protein levels in the CNS (Table 3.31), which can be related to dimecron-induced increment of RNA (Table 3.30).

Thus it can be concluded that the increase in the RNA concentration may be the result of possible selectively in protein synthesis (Prosser 1969), as well as diminished synthesis of RNA due to dimecron, poisoning.

4.9.0. Protein:

The total protein contents were reduced significantly in all the CNS regions of dimecron-intoxicated rats (Table

fish (Ravi 1984). Similar results were obtained in the CNS regions of fish brain following dichlorvos toxicosis (Rath and Misra 1980; Vadhva 1989). Methyl parathion has also been found to decrease the brain proteins (Khan 1989).

It is likely that dimecron inhibits the protein levels in the CNS probably by increasing the neuronal activity, and thereby enhancing the utilization of protein.

4.10.0 Accumulation of Dimecron in the CNS:

The nervous system is one of the most susceptible and vulnerable organs of the body (WHO 1986). Affecting the nervous system is therefore the quickest and most certain way of chemically upsetting the body metabolism. The biological action of most of the organophosphate pesticides is attributed to attacking the neuronal system, probably by penetrating the blood-brain barrier, and henceforth inhibiting cholinesterases which leads to disruption of neuronal transmission system (Paul et al 1979; Hall and Kolbe 1980). No report, however, is available till date, regarding accumulation of dimecron in the CNS following intoxication. The microanalysis of dimecron (Fig.3.9) accumulated in the CNS, is therefore of great importance for a better understanding of the OP neurotoxicity.

The present investigation discloses a remarkable dose related accumulation of dimecron in the cerebrum, cerebellum, brain stem and spinal cord of dimecron-treated rats (Table

3.32). A distinct dose-dependent increment in the concentration of dimecron was noticed in all the investigated regions of the CNS (Naqvi and Hasan 1990a).

Interestingly, in all the three dose groups (1.0, 1.5 and 2.0 mg / kg b.wt.), gray matter-rich regions viz. cerebrum and cerebellum showed higher uptake of dimecron compared to white matter rich regions, brain stem and spinal cord. This difference may be explained by the fact that the blood flow in the gray matter is many times higher than in the white matter (Landau et al 1955). This suggests that the gray matter is likely to be affected more rapidly by a toxicant, as compared to the white matter (Naqvi and Hasan 1990).

Highest percent uptake of dimecron was observed in the cerebrum in all the dose groups (22.2, 22.3 and 24.2%, respectively), while the lowest was noticed in brain stem (11.7, 11.4 and 12.0%). Notably, this finding is well in concordance with our earlier study (Naqvi et al 1988) where cerebrum showed maximum dimecron-induced depletion of phospholipids (57%), while the minimum depletion (-29%) was discernible in the brain stem. This indicates that dimecron toxicosis influences cerebrum more severely compared to other regions.

It can reasonably be concluded that dimecron penetrates the blood-brain barrier and exerts dose-dependent neurotoxic

effects through accumulation in the CNS, remarkably higher in the gray matter-rich regions than in the white matter-rich ones.

5. BIBLIOGRAPHY

1. Adams CWM. Nature 1961; **192**: 331.

2. Adrian ED, Feldberg W and Kilby BA. Brit J. Pharmacol. 1947; 2: 56.

3. Agnati LF, Benfenati F, Battistini N, Cavicchioli L, Fuxe K and Toffano G. Selective modulation of 3H-spiperone labeled 5-HT receptors by subchronic treatment with the ganglioside GM_1 in the rat. Acta Physiol Scand. 1983; 117: 311-314.

4. Agnati LF, Fuxe K, Toffano G, Galza L and Benfenati L, et al. Evidence for structural plasticity nigrostriatal dopamine neurons; Morphological evidence for trophic action of gangliosides on partially lesioned nigrostriatal dopamine neurons. In: Clinical Neuropharmacology. (Racagni G, Paoletti R and Kielholz P, eds.), Suppl I, Raven Press, New York, 1984; **7**: 594-595.

5. Agnati LF, Fuxe K, Benfenati F, Zoli M and Owman C, et al. Effect of ganglioside GM_1 treatment on striatal glucose metabolism, blood flow and protein phosphyration of the rat. Acta Physiol Scand. 1985; **125**: 43-53.

6. Ahmad IK, Begum MR, Sivaiah S and Ramana Rao KV. Effect of malathion on free amino acids, total proteins, glycogen and some enzymes of Pelecypod **Lamellidens marginalis** (Lamarck). Proc. Ind. Acad. Sci. Soc. B. 1978; 87: 377-380.

7. Al-Turk WA and Stohs SJ. Hepatic glutathione content and aryl hydrocarbon hydroxylase activity of acetaminophen-

treated mice as a function of age. Drug chem Toxicol. 1981; 4: 37-48.

8. Aldridge WN. The inhibition of cholinesterase by triesters of phosphoric acid. III. The nature of the inhibitory process. Biochem J. 1953; 54: 442-448.

9. Ali SF and Hasan M. Effect of organophosphate insecticide dichlorvos on the amino acid content of different regions of the rat brain and spinal cord. Indian J. Exp. Biol. 1977; 15: 759-761.

10. Ali, SF, Hasan M and Asifuzzaman. Observations on the organophosphate insecticide dichlorvos-induced changes in the amino acid and enzyme content of different regions of the central nervous system. J. Anat. Soc. India. 1977; 26: 12.

11. Ali, SF, Chandra O and Hasan M. Effect of an organophosphate (dichlorvos) on open field behaviour and locomotor activity; correlation with regional brain monoamine levels. Psychopharmacology. 1980; 68: 37-42.

12. Anliker R, Beriger E, Geiger M, and Schmid K. Uber die synthese von phosphamidon und seinen abbau in pflanzen. Helv Chim. Acta. 1961; 44: 1622-1645.

13. Anliker R and Menzer RE. Method of phosphamidon residue analysis. Agric Food Chem. 1963; 11: 291-293.

14. Arnaiz G, Rodrigues de Lores B, Robiolo de Esteves and Mistrorigo de Pacheco M. Inhibition of protein synthesis and ATPase in mitochondria by the administration of the convulsant 3-mercaptopropionic acid. Life Sci. 1975; 16: 385-394.

15. Avrova NF. Brain ganglioside patterns of vertebrates. J. Neurochem. 1971; **18**: 667-674.

16. Axelrod J. Noradrenaline: Fate and control of its biosynthesis. Science. 1971; **173**: 598-606.

17. Balareff D. Z Anorg Chem. 1917; **99**: 187.

18. Bami HL. Detection of pesticides in human poisoning. *Proceedings of First All India Symposium on Progress and problems in Pesticides Residue Analysis.* University of Ludhiana, Ludhiana 1971; p. 183.

19. Bano Y and Hasan M. Mercury-induced time-dependent alterations in lipid profiles and lipid peroxidation in different body organs of cat fish **Heteropneustes fossilis**. J. Environ Sci. Health. 1989; **B24 (2)**: 145-166.

20. Barrantes JF. Modulation of acetylcholine receptor states by thiol modification. Biochemistry. 1980; **19**: 2957-2965.

21. Beeman RW and Matsumura F. Chlordimeform: a pesticide acting upon amine regulating mechanisms. Nature. 1973; **242**: 273-274.

22. Bend JR, Smith BR, Ball LM and Mukhtar H. Alkene and arene oxide metabolism in hepatic and extrahepatic tissues - pharmacological and toxicological aspects. In: Conjugation Reactions in Drug Biotransformation (Aitio A, ed.) Biomedical Press, Elsvier, North Holland. 1978; p. 3-16.

23. Bergen WG, Czajka-Narins OM, Fink EL and Rimpau ES Liver and brain nucleic acids and brain RNA synthesis in suckling rats fed Dieldrin (38082). Proc. Soc. Exp. Biol. Med. 1974; **146**: 259-262.

24. Bergmann F, Rimon S and Segal R. Biochem J. 1958; 68: 493.

25. Bieri JG and Anderson AA. Arch Biochem Biophys. 1960; **90**: 105.

26. Bloor WR. Biochemistry of fatty acids and their compounds. In: The Lipids. Reinhold Publishing Corp., New York, 1943.

27. Bock E. Nervous system specific proteins. J. Neurochem. 1978; 30: 7-14.

28. Bon S, Vigny M and Massoulie J. A symmetric and globular forms of acetylcholinesterase in mammals and birds. Proc. Nat. Acad. Sci. (USA) 1979; **76**: 2546-2550.

29. Booth J, Boyland E and Sims P. Biochem J. 1961; **79**: 516.

30. Boyland E and Chasseaud LF. The role of glutathione and glutathione-S-transferases in mercapturic acid biosynthesis. Adv Enzymol. 1969; **32**: 172-219.

31. Boyland E and Williums K. Biochem J. 1965; **94**: 190.

32. Brante G. studies on lipids in the nervous system with special reference to quantitative chemical determination and topical distribution. Acta Physiol Scand. 1949; **18**: 63.

33. Bremer EG, Schlessinger J and Hakomori S. Ganglioside-mediated modulation of cell growth. J. Biol Chem. 1986; **261**: 2434-2440.

34. Brezezinsky J. The effect of poisoning with phosphorus organic insecticides on the catecholamine levels in rat plasma, brain and adrenals. Dissert Pharma Pharmacol. 1972; **24**: 217-220.

35. Brezezinski J. Effects of chlorfenuinfos on the disulfiram induced decrease of endogenous noradrenaline in the rat brain. Pol J. Pharmacol Phar. 1978; **30**: 69-71.

36. Brezezinski J and Paruszewska BW. Neurochemical alterations in rat brain as a test for study in the neurotoxicity of organophosphorus insecticides. Arch. Toxicol. Suppl. 1980; **4**: 475-478.

37. Burn JH and Rand NJ. AChE in adrenergic transmission. Ann Rev. Pharmacol 1965; **5**: 163.

38. Burton K. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J. 1956; **62**: 315-323.

39. Bus JS and Gibson JR. Lipid peroxidation and its role in toxicity. In: Reviews in Biochemical Toxicology. (Hodgson E, Bend JR and Philphot RM, eds). Elsevier, Amsterdam. 1979; p.125-149.

40. Caley RM and Jenson RG. Effect of parathion on lipolysis in isolation of rat adipose tissue cells. Lipids. 1973; **8 (1)**: 32-44.

41. Campbell PN. In: Progress in Biophysics and Molecular Biology. (Butter JAV and Huxley HE, eds). Pergamon Press, New York. 1965; **15**: p. 1-36.

42. Casida JE. Esterase inhibitors as pesticides. Science. 1964; **146**: 1011-1017.

43. Casida JE and Baron RL. Pesticide induced delayed neurotoxicity. US Environmental Protection Agency. Research Triangle Park, 1976.

44. Castillodel J and Kotz B. Prog Biophys Chem. 1957; **6**: 121.

45. Chadwick EL. Actions on insects and other invertebrates. In: Cholinesterases and Anticholinesterase Agents. (Koelle GB, ed). Handb Exp Pharmak, Springer-Verlag, Berlin 1963; **15**: 741-798.

46. Chambon P. Eucaryotic RNA polymerases. In: The Enzymes. (Boyer PD, ed.) 3rd ed., Academic Press, New York. 1974; **10**: p. 261-331.

47. Chambon P. Eukaryotic nuclear RNA polymerases. Annu Rev Biochem. 1978; **44**: 613-638.

48. Chase TN and Murphy DL. Serotonin and central nervous system function. Ann Rev Pharm. 1973; **13**:183-197.

49. Chasseaud LF. Extrahepatic conjugation with glutathione. In: Gram, Extrahepatic Metabolism of Drugs and Foreign Compounds. (SP Medical and Scientific Books, Jamaica). 1980; pp. 427-443.

50. Chio RS and Tappel AL. Inactivation of ribonuclease and other enzymes by peroxidizing lipids and malonaldehyde. Biochemistry. 1969; **8**: 2827-2832.

51. Chow CK and Tappel AL. An enzymatic protective mechanism against lipid peroxidation damage to lungs of ozone exposed rats. Lipids. 1972; **7**: 518.

52. CIBA monograph. Dimecron. (CIBA Ltd., Basle Switzerland). 1967 p. 29.

53. Clermont PH. Compt Rend. 1854; **39**: 338.

54. Collier B and Katz HS. The synthesis, turnover and release of surplus acetylcholine in a sympathetic ganglion. J Physiol (London). 1971; **214** : 537-552.

55. Collomp. Les Trilons, Bull. Inf. Scient. Min. Guerre (Sect. Techn. de l'Armee) Paris. 1949; **No.23 G**: 1.

56. Cooper JR, Bloor FF and Roth RH. The Biochemical Basis of Neuropharmacology. Oxford University Press, New York, 1978; pp. 320.

57. Costa E and Sandler M. Monoamine Oxidase: New Vistas. Raven Press, New York, 1972.

58. Coyle JT and Snyder SH. Catecholamines. In: Basic Neurochemistry. (Siegel GJ, Albers RW, Agranoff BW and Katzman R, eds). Little, Brown and Co., Boston 1981; p. 205.

59. Crapo JD, and Tierney DL. Superoxide dismutase and pulmonary oxygen toxicity. Am J. Physiol. 1974; **226**: 1401-1407.

60. Cumings JN. Brain 1955; **78**: 554.

61. Dahle LK, Hill EG and Holman RT, The thiobarbituric acid reaction and the autoxidation of polyunsaturated fatty acid methyl esters. Arch Biochem. 1962; **98**: 253-261.

62. Dandiya PC, Gupta BD and Gupta ML. Effects of L.S.D. on open field performance in rats. Psychoparmacologia. 1969; 15: 333.

63. Das M, Mukhtar H and Seth PK. Effect of acrylamine on brain and hepatic mixed function oxidases and glutathione-S-transferase(s) in rats. Toxicol Appl Pharmacol. 1982; 66: 420-426.

64. Davison AN and Dobbing J. Brain phospholipid metabolism in vivo. Biochem J. 1960; 71: 10.

65. Davison AN and Wajda M. Cerebral lipids in multiple sclerosis J. Neurochem. 1962; 9: 427.

66. De Lucia AJ, Haque PM, Mustafa MG and Cross CE. Ozone interaction with rodent lung. I. Effect on sulfhydryls and sulfhydryl-containing enzyme activities. J. Lab. Clin. Med. 1972; 80: 559.

67. Dean BJ. The effect of dichlorvos on cultured human lymphocytes. Arch Toxicol. 1972; 30: 75-85.

68. Demel RA, Somerharju P and Wirtz KWA. In: Phospholipid Metabolism (Horrocks LA, Kanfer JN & Porcellati G, eds). Raven Press, New York, 1984; Vol. II.

69. Demopoulos HB, Flamm ES, Seligman ML, Mitamura JA and Ransohoff J. Membrane perturbations in central nervous system injury: Theoretical basis for free radical damage and a review of the experimental data. In: Neural Trauma (Eds. Popp AJ, Bourke RS, Nelson LR, Kimelberg), Raven Press, New York, 1979; p. 63.

70. Department of Public Health, State of California. Reports of occupational disease attributed to pesticides and agricultural chemicals, Barkeley; 1957-1960.

71. Dische Z. Color reaction of nucleic acid components. In: (E. Chargaff and JN Davidson, Eds.) The Nucleic Acids, Vol. I. Acad Press, New York, 1955; p. 285-305.

72. Dixit R, Mukhtar H, Seth PK and Krishnamurty CR. Binding of acrylamide and glutathione-S-transferase. Chem Biol. Interact. 1980; **32**: 353-395.

73. Dixit R, Mukhtar H, Seth PK and Krishnamurti CR. Preliminary evidence for the presence of glutathione-S-transferase activity in mammalian and avian brain. Neurotoxicology 1981; **2**: 193-196.

74. Dobbing J. The entry of cholesterol into rat brain during development. J. Neurochem. 1963; **10**: 739-742.

75. DuBois KP, Doull J, Salerno PR and Coon JM. Studies on the toxicity and mechanism of action of p-nitrophenyl diethyl thionophosphate (parathion). J. Pharmacol Ther. 1949; **95**: 75-91.

76. Dudai Y, Herzberg M and Silman I. Molecular structures of acetylcholinesterase from electric organ tissue of the electric eel. Proc. Nat. Acad. Sci. (USA) 1973; **70**: 2473-2476.

77. Eccles JC. The physiology of nerve cells. The John Hopkins Press, Baltimore), 1957.

78. Edstrom JE. The content and the concentration of RNA in motor anterior horn cells from the rabbit. J. Neurochem. 1956; 1: 159-165.

79. Edstrom JE and Pigon A. Relation between surface, RNA content, and nuclear volume in encapulated spinal ganglion cells. J. Neurochem. 1958; 3: 95-99.

80. Elliott KAC and Heller IH. Metabolism of neuron and glia. *In: Metabolism of the Nervous System (Richter D, ed.)*. Pergamon Press, Oxford. 1957; p. 286-290.

81. Ellman GL, Courtney KD, Andre V Jr and Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961; 7: 88.

82. Emsley M, Gaines JB and Simon G. Toxicity of pesticides. Neurobiol Toxicol. 1976; 3(1): 36-38.

83. Enterbaver H. Aldehyde products of lipid peroxidation. In: Free Radicals, Lipid peroxidation and Cancer. (Mc Brien DCH and Slater TF, eds). Academic Press, New York 1982; p. 101-123.

84. Farahani H and Hasan M. Effect of NO_2 on lipids and lipid peroxidation in the CNS of the guinea-pig. Pharmacol & Toxicol. 1990; 66: 146-149.

85. Fiscus RR and Van Meter. Effects of parathion on turnover and endogenous levels of norepinephrine (NE) and dopamine (DA) in rat brain. Fed Proc. 1977; 36: 951.

86. Florkin M and Stolz EH. Comprehensive Biochemistry, Vol. 13. Report of the Commission on Enzymes of International

Union of Biochemistry. Elsevier Publishing Co., Amsterdam, London, New York, 1964.

87. Folch J, Ascoli I, Lees M, Meath JA and LeBaron FN. Preparation of lipid extracts from brain tissue. J. Biol Chem. 1951; **191**: 833-841.

88. Food and Agriculture Organization of the U.N./ World Health Organization. Evaluation of the toxicity of pesticide residues in food. Report of a joint meeting of the FAO committee on pesticides in agriculture and the WHO expert committee on pesticides residues. Geneva; 1964.

89. Francis BM, Hansen LG, Metcalf RA and Metcalf RL. Comparison of the delayed neurotoxicity of o-methyl and o-ethyl analogs of EPN, leptophos and cyanofenphos. J. Environ. Sci. Health (B). 1982; **17(6)**: 611-633.

90. Fridovich I. Accounts Chem. Res. 1972; 5: 321-325.

91. Fridovich I. Role of superoxide dismutase in defence against oxidative damage to the tissue. Ann Rev. Biochem. 1975; **44**: 147-159.

92. Fried RL. Monoamines. In: Topographic Brain Chemistry. Academic Press, New York and London, 1966, p. 366.

93. Fumagalli R and Paoletti R. The identification and significance of desmosterol in the developing human and animal brain. Life Sci. 1963; 5: 291.

94. Gitelson S, Davidson JT and Werczberger A. Phosphamidon poisoning. Brit J. Industr Med. 1965; **22** : 236-239.

95. Gregory EM and Fridovich I. Induction of superoxide dismutase by molecular oxygen. J. Bacteriol. 1973a; **114**: 543-548.

96. Gregory EM and Fridovich I. Oxygen toxicity and superoxide dismutase. J. Bacteriol. 1973 b; **114**: 1193-1197.

97. Gregory EM, Goscin SA and Fridovich I. J. Bacteriol. 1974; **117**: 456-460.

98. Grenell RG. Nucleic acid content of cerebral neurons. Neurology (Minneap.) Suppl. 8. 1958; **1**: 56-57.

99. Grover PL and Sims P. Conjugation with glutathione: Distribution of glutathione-S-transferases in vertebrate species. Biochem J. 1964; **90**: 603-606.

100. Gupta A and Hasan M. Age-related changes in lipid profiles and lipid peroxidation in the CNS following restraint stress. Indian J. Med. Res. 1988; **87**: 624-630.

102. Gupta BD, Dandiya PC, Gupta ML and Gabba AK. An examination of the effect of central nervous system stimulant and antidepressant drugs on open field performance in rats. Eur J. Pharmacol. 1971; **13**: 341-346.

103. Gupta PC. Neurotoxicity of chronic chlorinated hydrocarbon insecticide poisoning: A clinical study in man. Indian J. Med. Res. 1975; **63**: 901.

104. Gupta BD and Holland HC. An examination of the effects of stimulant and depressant drugs on escape-avoidance conditioning in strains of rats selectively bred for

emotionality-non-emotionality: a multivariate analysis of the effects of drugs on conditioned avoidance responses and intertrial activity. Neuropharmacology. 1972; **11**: 23.

105. Habig WH, Pabst MJ and Jakoby WB. Glutathione-S-transferases in mercapturic acid formation. J. Biol Chem. 1975; **249**: 7130-7139.

106. Haider SS, Hasan M, Hasan SN, Khan SR and Ali SF. Regional effects of sulfur dioxide exposure on the guinea pig brain lipids, lipid peroxidation and lipase Hactivity. Neurotoxicology. 1981; **2**: 443-450.

107. Haider SS, Hasan M and Khan NH. Air pollutant sulfur dioxide induced alterations in the levels of lipids, lipid peroxidation and lipase activity in various regions of the rat brain. Acta Pharmacol et toxicol. 1982; **51**: 45-50.

108. Haider SS and Hasan M. Neurobiochemical changes by inhalation of environmental pollutants sulfur dioxide and hydrogen sulfide: degradation of total lipids, elevation of lipid peroxidation and enzyme activity in discrete regions of the guinea pig brain and spinal cord. Industrial Health. 1984; **22**: 23-31.

109. Hall RJ and Kolbe E. Bioconcentration of organophosphorus pesticides to hazardous levels by amphibians. J toxicol Environ Health. 1980; **6**: 833-860.

110. Ham JM and Rose R. Platelet adhesiveness and lipoprotein lipase activity in controls and in subjects taking oral contraceptives. Am J. Obst Gynecol. 1969; **105**: 628-631.

111. Hasan M and Ali SF. Organophosphate pesticide dichlorvos-induced increase in the rate of lipid peroxidation in the different regions of the rat brain: supporting ultrastructural findings. Neurotoxicology. 1981; 2: 43-52.

112. Hasan M, Ali SF and Maitra SC. Electron microscope study of organophosphate DDVP-induced alterations in the rat hypothalamus and hippocampus. J Anat Soc India. 1979; 28: 117-120.

113. Hasan M and Khan NA. Methyl parathion induced dose-related alteration in lipid levels and lipid peroxidation in various regions of rat brain and spinal cord. Indian J Exp Biol. 1985; 23: 141-144.

114. Hasan M, Naqvi SMZ and Haider SS: Protective effect of cithiolone on the phosphamidon-induced increment of lipid peroxidation. VI Annual Conf. of Indian Acad Neurociences, held at Tirupati (AP, India), on March 4-8, 1987.

115. Haubrich DR and Chippendale TJ. Regulation of acetylcholine synthesis. Life Sci. 1977: 20: 1465-1478.

116. Hazzard WR, Spiger MJ, Bagdade JD and Bierman EL. Studies on the mechanism of increased plasma triglyceride levels induced by oral contraceptives. N Engl J Med. 1969; 280: 471-476.

117. Heath DF. Organophosphorus poisons, anticholinesterase and related compounds. Pergmon Press, Oxford, London, New York. 1961; p. 186.

118. Heikkila RE and Cohen G. Science 1973; **181**: 456-457.

119. Henly AA. The determination of serum cholesterol. Analyst. 1957; **82**: 286.

120. Hertz L. The biochemistry of brain tissue. In: The Biological Basis of Medicine (Bitter EE and Bitter N, eds). Academic Press, London & New York. 1969; pp. 3-37.

121. Hoch FL and Vallee BL. In: Sulfur in Proteins (Benesch R, Koltz JM, Benesch RE, Middlebook WR, Boyer PD and Schwarz DL eds). Academic Press, New York 1959; P. 245.

122. Holland HC and Gupta BD. Some correlated measures of activity and reactivity in two strains of rats selectively bred for differences in the acquisition of a conditioned avoidance response. Anim Behav. 1966; **14**: 574-580.

123. Holmstedt B. Pharmacology of organophosphorus cholinesterase inhibitors. Pharmacol Rev. 1959; **11(3)**: 567-588.

124. Holz RW. Evidence that catecholamine transport into chromaffin vesicles is coupled to vesicle membrane potential. Proc. Natl Acad. Sci (USA). 1978; **75**: 5190-5194.

125. Horrocks LA, Sun GY and Robert AD. Changes in brain during aging. In: Advances in Behavioral Biology; Neurobiology of Aging: An Interdisciplinary Life-span Approach. (Ordy JM and Brizzee KR, eds). Plenum Press, New York and London; 1975.

126. Hyden H. In: Brain Function (Brazier MAB, ed.). University of California Press, Berkeley and Los Angeles. 1964: 2: 29-68.

127. Hyden H and Lange PW. Protein changes in different brain areas as a function of intermittent training. Proc Natl Acad Sci (USA). 1972; 69(7): 1980-1984.

128. Indian Express. Pesticide poisoning in Punjab. July 22, 1987.

129. Irwin LN and Samson FE Jr. Content and turnover of gangliosides in rat brain following behavioral stimulation. J Neurochem. 1971; 18: 203-211.

130. Islam F, Tayyaba K and Hasan M. Organophosphate metasystox-induced increment of lipase activity and lipid peroxidation in cerebral hemisphere: diminution of lipids in discrete areas of rat brain. Acta Pharmacol et toxicol 1983; 53: 121-124.

131. Jokoby WB and Keen JH. A triple treat in detoxication by the glutathione-S-transferase. Trends Biochem Sci. 1977; 2: 229-232.

132. Jaques R and Bein H.J. Arch Toxikol, 1960; 18: 316.

133. Joh TH, Park DH and Reis DJ. Brain tryptophan hydroxylase: Purification of, production of antibodies to, and cellular and ultrastructural localization in serotonergic neurons of rat midbrain. Proc. Natl Acad Sci (USA). 1975; 72: 3575-3580.

134. Joh TH, Park DH and Reis DJ. Direct phosphorylation of brain tyrosine hydroxylase by cyclic AMP - dependent protein kinase: mechanism of enzyme activation. Proc Natl Acad Sci (USA). 1978; 75: 4744-4748.

135. Johnson AC. Concentration of lipids in the brain of infants and adults. Biochem J. 1949; 44: 494-498.

135b. Johnson MK. Biochem J. 1966; 98: 44.

136. Johnson MK. Mechanism of protection against the delayed neurotoxic effects of organophsophorus esters. Fed Proc. 1976; 35: 73-74.

137. Jorpes E. Biochem J. 1934; 28: 2102.

138. Kaloyanova FP, Zapryanov ZK and Baynova. Dokel Bolg Akad Nank. 1979; 32: 1153.

139. Kar PP and Matin MA. Duration of diazinon induced changes in the brain acetylcholine of rats. Pharmacol Res Commun. 1971; 3: 351-354.

140. Karpova OB, Obehova EL, Aviova NF and Schvart EG. Contents and composition of cerebral gangliosides during Down's Syndrome. Vopros Med Chim. 1978; 4: 524-527.

141. Kartha VNR and Krishnamurthy S. Factors affecting in vitro lipid peroxidation of rat brain homogenate. Int J. Physiol Pharmacol. 1978; 22: 44.

142. Katoh K, Konno N, Yamauchi T and Fukushima M. Effects of age on susceptibility of chickens to delayed neurotoxicity due to triphenyl phosphite. Pharmacol & Toxicol 1990; 66(5): 387-392.

143. Kaufman B, Basu S and Roseman S. Studies on the biosynthesis of gangliosides. In: Inborn Disorders of Sphingolipid Metabolism. (Volk BW and Aronson SM, eds.) Pergamon Press, Oxford 1967; p. 193-213.

144. Khan NA. The study of organophosphorus pesticide; alterations in the rat brain. Ph.D. Thesis. Aligarh Muslim University, Aligarh, 1989.

145. Khan NA and Hasan M. Dose-related neurochemical changes in the levels of gangliosides and glycogen in various regions of the rat brain and spinal cord following methyl parathion administration. Exp Pathol. 1988; **35**: 61-65.

146. Klien M and Frey HH. Central monoamines and convulsive thresholds in mice and rats. Neuropharmacol 1973; **12**: 681-692.

147. Klotzsche C. Neue insektizide phosphor-und phosphonsaureester. Nachr bl d deutsch Pfl sch dienstes. 1958; **10(4)**: 60-63.

148. Koelle GB. Citological distributions and physiological functions of cholinesterases. In: Cholinesterases and Anticholinesterase Agents. (Koelle GB, ed). Springer-Verlag, Berlin (suppl). 1963; **15**: 187-298.

149. Koenig E. Nucleic acid and protein metabolism of the axons. In: Handbook of Neurochemistry, Vol. 2. Plenum Press, New York. 1969.

150. Kulkarni SK and Dandiya PC. On the mechanism of potentiation of amphetamine-induced stereotyped

behaviour of imipramine. Psychopharmacologia. 1972; **27**: 367-372.

151. Laatsch RH, Kies MW, Gordon S and Alvord EC Jr. The encephalomyeltic activity of myelin isolated by ultracentrifugation. J Exp Med. 1962; **115**: 77-88.

152. Landa CA, Maccioni HJF and Caputto R. The site of synthesis of gangliosides in the chick optic system. J. Neurochem. 1979; **33**: 225-238.

153. Landau WM, Freygang WH Jr, Roland LP, Sokoloff L and Ketty SS. Advances in blood circulation of brain. Trans Amer Neurol Assoc. 1955; **80**: 125-131.

154. Landstrom H, Caspersson T and Wohlfart G. Uber den Nucleotidumstaz der Nervenzelle. Z. Zellforsch. 1941; **49**:534-548.

155. Lange W and Krueger GV. Ber. 1932; **65**: 1598.

156. Lassaigne IL. Ann Chimie Physique. 1820; **13** (2 Serie): 294.

157. Lat J. The spontaneous explorating reactions as a tool for psychopharmacological studies: a contribution towards a theory of contradictory results in psychoparmacology. Proceedings of 2nd International Pharmacological Meeting, 22-23 August 1963, Praha (Czechoslovak Medical Press, Prague), 1965, p.47.

158. Lauwerys R and Buchet JP. Studies on the mechanism of toxicity of the organophosphorus pesticide triamiphos. Eur J Pharmacol. 1971; **16**: 361-366.

159. Ledeen RW. Gangliosides structure and distribution: Are they localized at the nerve endings? J Supramol Struct. 1978; 8: 1-17.

160. Ledeen WR and Mellanby J. Gangliosides as receptors for bacterial toxins. In: Perspectives in Toxicology (Bernheimer AW, ed.) Wiley, New York. 1977; p. 15-42.

161. Ledeen RW, Skriyanek JA, Tirri LJ, Margolis PK and Margolis RV. Gangliosides function. In: Biochemical and Pharmacological Implications (Porcellati G, Ceccareti B and Tettamanti G, eds). Adv Exp Med Biol. Plenum Press, New York, 1976; 71: 83-103.

162. Ledeen R and Yu R. Structure and enzymatic degradation of sphingolipids. In: Lysosomes and Storage Diseases (Hers HG and Van Hof F, eds.). Academic Press, New York, 1973; p. 105-145.

163. Lehman AJ. Summaries of pesticide toxicity. The association of food and drug officials of the United States. Topoka, Kansas; 1965.

164. Levene PA and Jacobs WA. Ber. 1909; 42: 2102.

165. Levene PA, Mikeska LA and Mori T. J. Biol Chem. 1930; 85: 785.

166. Lichtentahler FW. The chemistry and properties of enol phosphates. Chem Rev. 1961; 61: 607-649.

167. Little C and O'Brien PJ. An intracellular GSH peroxidase with a lipid peroxide substrate. Biochem Biophys Res Commun. 1968; 31: 145-157.

168. Logan JE. Mannell WA and Rossiter RJ. Estimation of

nucleic acids in tissue from the nervous system. Biochem J. 1952; 51: 470-479.

169. Lohs KH. Synthetische Gifte. 3rd Edition. Deutscher Militarverlag, Berlin. 1967; p. 190, 204, 248.

170. Lorenz W. (Farbenfabriken Bayer AG). D.B.P. 1.003.720; (1954/1957).

171. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin-phenol reagent. J. Biol Chem. 1951; 3: 193-265.

172. Mahler HR. Nucleic acid and protein metabolism. In: Basic Neurochemistry. (Siegel GJ, Albers RW, Agranoff BW & Katzman R, eds.) 3rd edition, Little - Brown & Co., Boston. 1981; p. 371-400.

173. Majno G and Karnovsky ML. Respiration and lipid biosythesis by rat nerves **in vitro**, after treatment with cholinesterase inhibitor (Mipafox). Fed Proc. 1955; **4**: 250.

174. Majno G and Karnovsky ML. A biochemical and morphologic study of myelination and demyelination. III. Effect of an organophosphorus compound (Mipafox) on the biosynthesis of lipids by nervous tissue of rats and hens. J Neurochem. 1961; 8: 1-9.

175. March RB, Fukuto TR, Metcalf RL and Maxon MG. Fate of 32_p labelled malathion in the laying hen, white mouse and American cockroach. J Econ Entomol. 1956; **49**: 185-195.

176. Marinetti GV. Chromatographic separation, identification and analysis of phosphatides. J Lipid Res. 1962; 3: 1-20.

177. Marklund S and Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974; 47: 469-474.

178. Massey V, Strickland S, Mayhew SG, Howell LG, Engel PC, Matthews RG, Schuman M and Sullivan PA. Biochem Biophys Res Commun. 1969: 36: 891-897.

179. Mattson AM, Spillane JT, and Pearce CW. J Agr Food Chem. 1955; 3: 319.

180. May L and Grenell RG. Nucleic acid content of various areas of the rat brain. Proc. Soc . Exp Biol Med. 1959; 102: 235-239.

181. McCord JM and Fridovich I. Fed Proc. 1969: 28: 346.

182. McIlwain H and Bachelard HS. Biochemistry and the central nervous system. Churchill Livingston, London; 1971.

183. Mead JF. Free radical mechanisms of lipid damage and consequences for cellular membranes. (Pryor WA, ed). 1976.

184. Michael JB. Serotonin, histamine, and the purines. In: Basic Neurochemistry. (Siegel GJ, Albers RW, Agranoff BW and Katzman R, eds). Little, Brown and Co., Boston. 1981; p. 205.

185. Menzer RE and Best NH. Effect of phenobarbital on the toxicity of several organophosphorus insecticides. Toxicol Appl Pharmacol. 1968; **13**: 37-42.

186. Michaelis A. Liebigs Ann Chem. 1903; **326**: 172.

187. Michaelis A and Becker TH. Ber. 1897; **30**: 1003.

188. Michaelis A and Kaehna R. Ber. 1898; **31**: 1048.

189. Michelson AM and Buckingham ME. Effect of superoxide radicals on myoblast growth and differentiation. Biochem Biophys Res Commun. 1974; **58**: 1079-1086.

190. Miescher F. Hoppe-Seyler's Med Chem. Unters. 1871; p. 441.

191. Michailovic L, Jankovic BD, Petkovic M and Mancic D. Distribution of DNA and RNA in different regions of cat's brain. Experimentia. 1958; **14**: 9-10.

192. Ministry of Health and Welfare, Japanese Govt. OP Poisoning. Tokyo . 1970; p. 170.

193. Misra HP and Fridovich I. The role of superoxide anion in autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; **247**: 3170-3175.

194. Moriarty F. Pollutants and animals: a factual perspective. George Allen and Unwin Ltd, London. 1975.

195. Mukhtar H, Zoetmelk CEM, Baars AJ, Wijnen JTH, Wijnen LMMB and Briemer DD. Glutathione-S-transferase in human fetal and adult tissues. Pharmacology. 1981; **22**: 322-329.

196. Murphy SD. Pesticides. In: Casarett and Doull's Toxicology-The Basic Science of Poisons. (Doull J, Klaassen CD and Arndur MO, eds). MacMillan Publications, New York. 1980; pp. 357-408.

197. Murphy SD, Lawverys RL and Cheever KL. Comparative anticholinesterase action of organophosphorus insecticides on vertebrates. Toxicol Appl Pharmacol. 1968; 12: 22-35.

198. Muthazhagu S. Neurological consequences of dimethoate, an organophosphate pesticide in the rat, **Rattus norvegicus**. Ph.D. thesis. University of Madras, Madras, 1985.

199. Nag A and Ghosh JJ. Sumithion induced neurotoxicity in pigeons: Effect on lipid metabolism of spinal cord. Neurosci Lett. 1984; **46**: 335-339.

200. Nagaratnama R and Ramamurthy R. **In vivo** recovery of acetylcholinesterase activity from methyl parathion induced inhibition in the fresh water teleost **Cyprinus capio**. Curr Sci. 1983; **52**: 74-75.

201. Naqvi SMZ and Hasan M. Dose-related accumulation of organophosphate phosphamidon in discrete regions of the CNS: correlation with its neurotoxicity. Pharmacol & Toxicol. 1990; **67(1)**: 47-48.

202. Naqvi SMZ and Hasan M. Phosphamidon neurotoxicity: Protection by acetylhomocysteine thiolactone. NeuroReport (Oxford). 1991 a; **2(1)**: 61-63.

203. Naqvi SMZ and Hasan M. Regional alteration in the monoamine levels, monoamine oxidase activity and

neurobehavioral effects in rats treated with dimecron (phosphamidon). Indian J Med Res (B). 1991 b; **94**: 71-75.

204. Naqvi SMZ and Hasan M. Neurotoxic effects of organophosphate dimecron on monoamine neurotransmitters: correlation with acetylcholinesterase activity. In: Biomembranes in Health and Disease, Vol. 1 (Kidwai AM, Upreti RK & Ray PK, eds), Today and Tomorrow's Printers and Publishers, New Delhi. 1991 c; p. 277-280.

205. Naqvi SMZ, Hasan M and Haider SS. Neurochemical effects of phosphamidon on lipid fractions and lipid peroxidation in various regions of the rat central nervous system. Indian J Med Res. 1988; **88**: 85-90.

206. Neame KD. Transport, metabolism and pharmacology of amino acids in brain. In: Applied Neurochemistry. (Davison AN & Dobbing J, eds). Blackwell Scientific Publication, Oxford. 1968; p. 119-120.

207. Nelson WL and Barnum CP. Effect of diisopropyl phosphorofluoride on mouse brain phosphorus metabolism. J Neurochem. 1960; **6**: 43-50.

208. North EA, Pawlyszyn G and Doery HM. The action of phosphatidase A, sodium oleate and ganglioside on the exotoxins of Cl. Welchil and on the neurotoxin of Shigella Shigae. Aust J Exp Biol. 1961; **39**: 259-266.

209. Norton WJ and Poduslo SE. Myelination in rat brain: changes in myelination composition during brain maturation. J Neurochem. 1973; **21**: 759-773.

210. Nylen P. Studien uber organische phosphorverbindungen (Inaugural Dissertation, Uppsala), 1930.

211. O'Brien RD. Toxic Phosphorus Esters. Academic Press, New York; 1960; pp. 89-93.

212. O'Brien RD. Acetylcholinesterase and its inhibition. In: Insecticide Biochemistry and Physiology. (Ed. Wilkinson CF, ed). Plenum Press, New York. 1976, pp. 271-296.

213. Ordy JM and Kaack B. Neurochemical changes in composition, metabolism and neurotransmitters in human brain with age. In: Neurobiology of Aging. (Ordy JM and Brizzee KR, eds). Plenum Press, London & New York, 1975; p. 264.

214. Palanivelu K. Neurochemical and neurophysiological alterations induced by phosalone, an organophosphate pesticide, in the fore-brain of male albino rat, **Rattus norvegicus**. M. Phil dissertation, University of Madras, 1984.

215. Paoletti R, Fumagalli R and Grossi E. Studies in brain sterols in normal and pathological conditions. J Am Oil Chem Soc. 1965; **42**: 400-404.

216. Paolo P and Fini A. Damage by dichlorvos of human lymphocyte DNA. Tumori. 1960; **66**: 425-430.

217. Parale MP and Kulkarni SK. Clonidine induced behavioural despair in mice: reversal by antidepressants. Psychopharmacology. 1986; **89**: 171.

218. Patridge CA, Dao DD, Hong TD, Misra G, Polse DS and Awasthi YC. The effect of t-butylated hydroxytoluene on glutathione linked detoxication mechanism in rats. Arch Toxicl. 1983; 53: 427-437.

219. Paul BS, Gupta RC and Malik JK. Influence of phenobarbitone and atropine on malathion induced toxicity and related biochemical changes in rats. Indian J Exp Biol. 1979; **17**: 1096-1099.

220. Peng SK, Taylor CB, Hill JC and Morin RJ. Cholesterol oxidation derivations and arterial endothelial damage. Atherosclerosis. 1985; **54**: 121-133.

221. Perkow W. Ber. 1954; **87**: 755.

222. Perkow W, Ullerich K and Meyer F. Naturwissenschaften. 1952; **39**: 353.

223. Pollet S, Ermidou S, Lesaud F, Mange M and Baumann N. Microanalysis of brain lipids: multiple two-dimentional thin layer chromatography. J Lipid Res. 1978; **19**: 916-921.

224. Porcellati G. Neural Membranes. (Sun GY, Bazan N, Porcellati G & Sun AY, eds). Humana Press, Clifton, New Jersy. 1983; p. 3-35.

225. Preti A, Fiorilli A, Lombardo A, Caimi L and Tettamanti G. Occurance of sialyltransferase activity in the synaptosomal membranes prepared from calf brain cortex. J Neurochem. 1980; **35**: 281-296.

226. Prosser CL. Principles and general concepts of adaptation. Environ Res. 1969; **2**: 404-416.

227. Pryor WA, Stanley JP and Blair E. Autoxidation of polyunsaturated fatty acids II: a suggested mechanism for the formation of TBA - reactive material from prostaglandin-like endoperoxides. Lipids. 1976; II: 370-379.

228. Rahman H. Functional implications of gangliosides in synaptic transmission. A Critique Neurochem Inter. 1983; 5: 539-547.

229. Rahman H, Probst W and Muhleisen M. Gangliosides and synaptic transmission. Jpn J Exp Med. 1982; **52**: 275-286.

230. Rahman H and Rosner H. Autoradiographus due untersuchungen Zun Einbauvon N-acetyl-3H-mannosamin im optischen system. Von Telcosteen Z Zell torsch. 1973; **141**: 93-101.

231. Rajagopalan KV, Fridovich I and Handler P. J Biol Chem. 1962; 237: 922-928.

232. Rajagopalan KV and Handler P. J Biol Chem. 1964; **239**: 2022-2026.

233. Rao KSP and Rao KVR. Changes in the tissue lipid profiles of fish (**Oreochromis mossambicus**) during methyl parathion toxicity - A time course study. Toxicol Lett. 1984; 21: 147-153.

234. Rath S and Misra BN. Changes in nucleic acids and protein content of **Tilapia mossambica** exposed to Dichlorvos (DDVP). Indian J Fish. 1980; **27(1/2)**: 76-81.

235. Ravi G. Studies on neurochemical and neurophysiological alterations in the two species of edible fish, **Labeo rohita** (HAM) and **cyprinus carpio communis** (LINN) exposed to phosalone, an organophosphate pesticide. Ph.D. Thesis, University of Madras, 1984.

236. Rieger F, Bon S, Massoulie and Cartaud J. Observation par microscopie electronique des formes allongees et globulares des lacetycholine de gymnote. Eur J Biochem. 1973; **34**: 539-547.

237. Reiner E. Spontaneous reactions of phosphorylated and carbomylated cholinesterase. Bull WHO. 1971; **44**: 109-112.

238. Richardson PJ, Walker JH, Jones RT and Whittaker VP. Identification of a cholinergic - specific antigen Chol-1 as a gangliosides. J Neurochem. 1982; **37**: 1605-1614.

239. Riser A. Protar. 1950; **16(11/12)**: 132.

240. Roberts J, Freysz L, Sensenbrenner M, Mandel P, and Rebel G. Gangliosides of glial cells: a comparative study of normal astroblasts in tissue culture and glial cells isolated on sucrose-Ficoll gradients. FEBS Lett. 1975; **50**: 144.

241. Rosin J and Haus J. (Montrose Chem. Comp.) A.P. 2.899.456 (1956/1959).

242. Rossiter RJ. Biosynthesis of phospholipids and sphingolipids in the nervous system. In: Nerve as a Tissue. (Rodahl K and Issekutz B, eds). Harper and Row, New York. 1966; p. 175-194.

243. Saavedra JM, Brownstein M and Axelrod J. A specific and sensitive enzymatic-isotopic microassay for serotonin in tissues. J Pharmacol Exp Ther. 1973; **186**: 508-515.

244. Sandhoff K and Christomanoue H. Biochemistry and genetics of gangliosidosis. Hum Genet. 1979; **50**: 107-143.

245. Sasaguri Y, Nakashima T, Marimatsu M and Yagi K. Injury to cultured endothelial cells from human umbilical vein by lineolic acid hydroperoxide. J Appl Biochem. 1984; **6**: 144-150.

246. Scharf JH. Die Bezichungen der Lipoide zu de Perizelluren der Ganglienzellen bei einingen Wirbellosen im Vergleich Zu Wirbeltiern. Z Zellforsch. 1953; **38**: 526-570.

247. Scheckel CL, Boff E an Pazery LM. Hyperactive states related to the metabolism of norepinephrine and similar biochemicals. Ann NY Acad Sci. 1969; **159**: 939-958.

248. Schimizu Y. Note on myelin formation around statoacoustic ganglion cells cultivated **in vitro**. Acta Anat Nippon. 1965; **40**: 138-139.

249. Schmidt G. Nucleases and enzymes attacking nucleic acid components. In: The Nucleic Acids: Chemistry and Biology. Vol. 1 (Chargaff E and Davidson JN, eds.). Academic Press Inc, Publishers, New York. 1955; p. 555-626.

250. Schrader G. (Farbenfabriken Bayer AG). D.B.P. 767.511 (1937/1952).

251. Schrader G. BIOS Final Report. 1949; **949**: 6.

252. Schrader G. Die Entwicklung neuer Insektizide auf Grundlage von organischen Fluor-und

phosphorverbindungen. Monographic No.62. Verlag Chemic, Weinheim; 1952.

253. Schrader G and Gebhardt H. (Farbenfabriken Bayer AG). D.B.P. 767-830 (1939/1953).

254. Searchy DG and Maclnnis AJ. Determination of RNA by Dische-orcinol techniques. In: Experiments and techniques in parasitology. (Maclnnis AJ and Voge M, eds.) WH Freeman and Co, Sanfrancisco. 1970; p. 189-190.

255. Sedlak J and Lindsay RH. Estimation of total, protein-bound and non-potein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem. 1968; **25**: 192-205.

256. Shallenberger MK and Walaszec EJ. Pharmacology of central nervous system. Progr Neurol Psychiat. 1972; **27**: 67-86.

257. Singer SJ and Nicolson GL. The fluid mosaic model of the structure of cell membranes. Science. 1972; **175**: 720-731.

258. Skau KA and Brimijoin S. Release of acetylcholinesterase from rat hemidiaphragm preparation stimulated through the phrenic nerve. Nature. 1978; **275**: 224-226.

259. Smith EL, Hill RL, Lehman JR, Lefkowitz RJ, Handler P and White A. The nervous system. In: Principles of Biochemistry; Mammalian Biochemistry. Chapter 7, 7th Edition. McGraw Hill International Book Company, Tokyo. 1983.

260. Smith GJ, Ohl VS and Litwack G. Ligandin, the glutathione-S-transferase and chemically induced

hepatocarcinogenesis: A review. Cancer Res. 1977; **37**: 8-14.

261. Smith ME, Fumagalli R and Paoletti R. The occurrance of desmosterol in myelin of developing rats. Life Sci. 1967; **6**: 1085-1091.

262. Sobrino JA and Del Castillo J. Activation of the cholinergic end-plate receptors by oxidizing reagents. Int J Neurosci. 1972; **3**: 251-258.

263. Sokoloff L. Mapping of local cerebral functional activity by measurement of local cerebral glucose utilization with (14C) deoxyglucose. Brain. 1979; **102**: 653.

264. Soto EF, de Bohner SL and Calvino M del C. Chemical composition of myelin and other subcellular fractions isolated from bovine white matter. J Neurochem. 1966; 13: 989.

265. Stanmbolova MA, Cox D and Mathias AD. Biochem J. 1973; **136**: 685-695.

266. Stockstill ME and Dauterman WG. Studies on the induction of glutathione-S-transferase in liver. Drug and Chem Toxicol, 1982; 5: 427-437.

267. Stokinger HE and Coffin DL. Biological effects of air pollutants. In: Air pollution (ed. Stern AC). 2nd Edition, Academic Press, New York, 1968; p. 445.

268. Suzuki K. Chemistry and metabolism of brain lipids. In: Basic Neurochemistry. (Siegel GH, Albers RW, Agranoff BW and Katzman R, eds). 3rd edition, Little-Brown & Co., Boston. 1981; p. 355-371.

269. Svennerholm L. Gangliosides and synaptic transmission. In: Advances in Experimental Biology and Medicine; Structure and Function of Gangliosides. (Svennerholm L, Mandel P, Dreyfus H and Urban P eds.) Plenum Press, New York. 1980; **125**: 533-544.

270. Tabershaw IR and Cooper WC. Sequelae of acute organophosphate poisoning. J Occup Med. 1966; **8**: 5.

271. Tabor CW, Tabor H and Rosenthal SM. Monoamine oxidase. Methods Enzymol. 1953; **2**: 390.

272. Tamilvanan R. Poisonous effects of organophosphate pesticide phosalone on the neurochemical aspects of related regions of male albino rat brain. M. Phil. dissertation, University of Madras, 1984.

273. Tanford C. The hydrophobic effect and the organization of living membranes. Science. 1978; **200**: 1012-1018.

274. Tappel AL. Lipid peroxidation damage to cell components. Fed Proc. 1970; **29**: 239.

275. Tappel AL and Dillard CJ. In vivo lipid peroxidation: measurement via exhaled pentane and protection by vitamin E. Fed Proc. 1981; **40**: 174.

276. Taylor KM and Snyder SH. Differential effects of d- and l- amphetamine on behaviour and on catecholamine deposition in dopamine and norepinephrine - containing of rat brain. Brain Res. 1971; **28**: 295-309.

277. Tayyaba K and Hasan M. Organophosphate insecticide DDVP-induced increment in regional total lipids and cholesterol levels: diminution of phospholipid

concentration in different regions of the rat brain. Current Sci. 1980; **49**: 617-619.

278. Tayyaba K and Hasan M. Vitamin E protects against metasystox-induced adverse effects on lipid metabolism in the rat brain and spinal cord. Acta Pharmacol et toxicol. 1985; 57: 190-196.

279. Tayyaba K, Hasan M, Islam F and Khan NH. Organophosphate pesticide metasystox - induced regional alterations in brain nucleic acid metabolism. Indian J Exp Biol. 1981; 19: 688-690.

280. Tien M, Svingen BA and Aust SD. Superoxide dependent lipid peroxidation. Fed Proc. 1981; 179-182.

281. Toivonen T, Ohela K and Kaipaionen WJ. parathion poisoning increasing frequency in Finland. Lancet. 1959; 2: 175.

282. Totaro AE, Pisanti FA, Continillo A, and Liberatori E. Morphological evaluation of the lipofuscinolytic effect of acetylhomocystein thiolactone. Arch Gerontol Geriatr. 1985; **4**: 67-72.

283. Tracey JA and Gallagher H. Use of glycopyrrolate and atropine in acute organophosphorus poisoning. Hum Exp Toxicol. 1990; **9(2)**: 99-100.

284. Tracy RL, Woodcock JG, and Chodroff S. Toxicological aspect of 2,2-dichlorovinyl-dimethyl-phosphate (DDVP) in cows, horses and white rats. J Econ Entomol. 1960; 53: 593-601.

285. Tsumuki H, Saito T and Miyata T. Acute and subacute toxicity of organophosphorus insectides to mammals. In:

Biochemical Toxicology of Insecticides (O'Brien RD and Yamamoto I, eds.). Academic Press, New York 1970; pp. 65-73.

286. Utley HC, Bernheim F and Hochstein P. Effect of sulfhydryl reagents on peroxidation in microsome. Arch Biochem Biophys. 1967; **118**: 29-32.

287. Vadhva P. Neurotoxicity of an organophosphorus compound (dichlorvos) in a teleost. Ph.D. Thesis. Aligarh Muslim University, Aligarh, 1989.

288. Vadhva P and Hasan M. Organophosphate dichlorvos induced dose-related differential alterations in lipid levels and lipid peroxidation in various regions of the fish brain and spinal cord. J Environ Sci Health. 1986; **B21 (5)**: 413-424.

289. Vasilescue C, Alexianu M and Dan A. Delayed neuropathy after organophosphorus insecticide (Dipterex) poisoning: a clinical, electrophysiological and nerve biopsy study. J Neurol Neurosurg Psychiatry. 1984; **47(5)**: 543-548.

290. Vercruysse A and Deslypere P. J Forens Med. 1964; II : 107.

291. Vladimerov Yu A and Archakov AI. Perekisnoji Okislenijie Lipidov-V-Bilogiches Kikh Mennransikh. "Nauka", Moscow. 1972.

292. Voss G. The fundamental kinetics of cholinesterase reactions with substrates and inhibitors in an automated continuous flow system. In: Residue Reviews. Vol. 23 (Gunther FA, ed), Springer Verlag, Berlin, 1968; p. 112.

293. Waelsch H, Sperry WM and Stayanoff VA. A study of the synthesis and deposition of lipids in brain and other tissues with deuterium as an indicator. J Biol Chem. 1940; 135: 291-296.

294. Weiss CM. Physiological effect of organic phosphorus insecticides on seven species of fish. Trans Amer Fish Soc. 1959; **90**: 143-152.

295. Weiss LR, Bryant J and Pitzhugh CG. Blood sugar levels following acute poisoning with parathion and 1-naphthyl-N-methyl-carbamate (Sevin) in three species. Toxicol Appl Pharmacol. 1964; 6: 363-364.

296. Welch AS and Welch BL. Solvent extraction method for simultaneous determination of norepinephrine, dopamine, serotonin and 5-hydroxyindoleacetic acid in a single mouse brain. Anal Biochem. 1969; **30**: 161-169.

297. Wells ED and Dittmer JC. A comprehensive study of the post-natal changes in the concentration of the lipids of developing rat brain Biochemistry. 1967; **6**: 3169-3175.

298. White A, Handler P, Smith EL and and Stellen DW Jr. In: Principles of Biochemistry. 2nd Ed. McGraw-Hill Inc., New York. 1959; p. 458.

299. White A, Handler P, Smith EL, Hill RL and Lehman IR. The lipid metabolism. In: Principles of Biochemistry, 6th ed. McGraw-Hill and Kogakusha Ltd., New York and Tokyo. 1978; p. 38-1133.

300. Wilson IB. Acetylcholinesterase XI. Reversibility of tetraethyl pyrophosphate inhibition. J Biol Chem. 1951' **190**: 111-117.

301. Wilson IB. The mechanism of enzyme hydrolysis studied with acetylcholinesterase. In: Symposium on the mechanism of Enzyme Action. (McElroy WE and Glass B, eds). The Johns Hopkins Press, Baltimore, 1954; pp. 642-657.

302. Wilson IB and Bergmann F. Acetylcholinesterase VIII. Dissociation constants of the active groups. J Biol Chem. 1950; **186**: 683-692.

303. Wirth W. Arch Exp Pathol Pharmakol, 1949; **207**: 545.

304. Wolfe SL. Methods for separation and determination of gangliosides. In: Research Methods in Neurochemistry. (Marks N and Rodnight R, eds). Plenum Press, New York, and London. 1972.

305. Wood JL. In:Metabolic conjugation and Metabolic Hydrolysis (Fishman WH, ed), Vol. 2, Academic Press, New York. 1970; p. 261-299.

306. Woodman DD and Price CP. Estimation of serum total lipid. Clinica Chim Acta. 1982; **38**: 39-43.

307. World Health Organization. Toxic hazards of pesticides to man. 12th Report of the Expert Committee on Insecticides. Technical Report Series No. 227; 1962.

308. World Health Organization. Principles and methods for the assessment of neurotoxicity associated with exposure to chemicals. Env Health Criteria, No. **60**, WHO, Geneva. 1986; p. 110-119.

309. Yu RK and Iqbal K. Sialosylgalactosyl ceramiole as a specific marker for human myelin and oligodendroglia and neurons. J Neurochem 1979; **32**: 293-300.

PUBLICATIONS AND PRESENTATIONS

Publications

1. **Naqvi SMZ**, Hasan M and Haider SS. Neurochemical effects of phosphamidon on lipid fractions and lipid peroxidation in various regions of the rat central nervous system. Indian J Med Res. 1988; 88: 85-90.

2. **Naqvi SMZ**, Haider SS, Hasan M and Mahdi A. Dimecron induced decrement in the levels of total lipids, phospholipids, cholesterol and gangliosides in various regions of the rat CNS. Proc. Annual Conference of Anat Soc. India. J Anat Soc India. 1987: 68.

3. **Naqvi SMZ** and Hasan M. Dose-related accumulation of organophosphate phosphamidon in discrete regions of the CNS: Correlation with its neurotoxicity. Pharmacol & Toxicol (Denmark). 1990; 67: 47-48.

4. **Naqvi SMZ** and Hasan M. Phosphamidon neurotoxicity: Protection by acetylhomocysteine thiolactone. NeuroReport (Oxford) 1991a; 2(1): 61-63.

5. **Naqvi SMZ** and Hasan M. Regional alterations in the monoamine levels, monoamine oxidase activity and neurobehavioral effects in rats treated with dimecron (Phosphamidon). Indian J Med Res [B] 1991 b; 94: 71-75.

6. **Naqvi SMZ** and Hasan M. Neurotoxic effects of organophosphate dimecron on the monoamine neurotrasmitters: Correlation with acetylcholinesterase activity. In: Biomembranes in Health and Disease. Vol 1 (AM Kidwai, RK Upreti & PK Ray, eds). Today & Tomorrow's Printers & Publishers, New Delhi, 1991c; p. 277-280.

7. **Naqvi SMZ** and Hasan M. Acetylhomocysteine thiolactone protects against dimecron-induced perturbations in the open field behaviour and MAO activity of the CNS. Pharmacol & Toxicol. 1991 (Communicated).

8. **Naqvi SMZ** and Hasan M. Neurotoxic effect of dimecron on superoxide dismutase and lipid peroxidation: Protection by acetylhomocysteine thiolactone. Neurotoxicology. 1991 (Communicated).

Presentations:

1. Hasan M, **Naqvi SMZ** and Haider SS. Protective effect of cithilone on the phosphamidon-induced increment of lipid peroxidation, 6th Conference of Indian Academy of Neurosciences, held at Tirupati on March 4-5, 1987.

2. **Naqvi SMZ** and Hasan M. Neurotoxic effects of organophosphate dimecron on brain nucleic acids and

protein metabolism. 7th Conference of Indian Acad Neurosciences, held at Calcutta on March 7-8, 1988.

3. Mahdi AA, Ahmad S, Khan HM, Khanna R and **Naqvi SMZ.** Effect of **Plasmodium berghei** on the lipid fractions and lipid peroxidation in discrete regions of mice brain. 7th Conference of Indian Acad Neurosciences, held at Calcutta on March 7-8, 1988.

4. Hasan M, Ali SF, Islan F, Tayyaba K, Vadhva P, Khan NA and **Naqvi SMZ.** Neurotoxicity of organophosphate pesticides: Contribution of the past decade. First Afro-Asia Oceania Congress of Anatomists (AAOCA), held at All India Institute of Medical Sciences, New Delhi, on August 29- September 3, 1988.

5. **Naqvi SMZ** and Hasan M. Dimecron-induced neurotoxic effects on the contents of sulfhydryl radicals: a correlation with enhanced lipid peroxidation. 8th Conference of Indian Acad Neurosciences, held at PGIMER, Chandigarh on March 24-26, 1989.

6. Hasan M and **Naqvi SMZ.** Dose-related accumulation of dimecron in the CNS: Correlated with its neurotoxicity. 12th Conference of UP Chapter of Anatomical Society of India, held at AMU, Aligarh on December 9-10, 1989.

7. **Naqvi SMZ** and Hasan M. Neurotoxic effects of organophosphate dimecron on regional MAO activity and biogenic amines: Correlated with accumulation of dimecron in various CNS regions. 9th Conference of Indian Acad Neurosciences, held at ITRC, Lucknow, on March 16-18, 1990.

8. **Naqvi SMZ** and Hasan M. Acetylhomocysteine thiolactone protects against phosphamidon-induced depletion of glutathione-S-transferase in the CNS: Correlated with perturbed sulfhydryl groups. 10th Conference of Indian Acad Neurosciences and 7th meeting of the Neurosciences Society of India; helt at JNU, New Delhi on December 12-14, 1990.